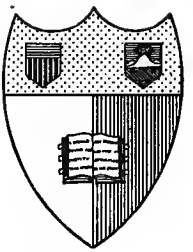

PHYSICAL CHEMISTRY

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ITS BEARING ON BIOLOGY AND MEDICINE

BY

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PREFACE

THE conceptions and methods of physical chemistry have been extensively utilised during recent years in attacking biological and physiological problems, and the results which have followed this application of physico-chemical principles are not only of very great interest in themselves, but are full of promise for the future. Students of biology and medicine, however, cannot truly appreciate or co-operate in this work unless they are familiar with the underlying principles. It is as a sketch of the physico-chemical basis for this modern treatment of biological and physiological problems that I have written the present volume.

The book originated in a course of lectures delivered to biological students at the University of London in 1909, but since then much fresh material has been incorporated. I have endeavoured to give a systematic exposition of physical chemistry so far as it has a bearing on the problems in question, and to illustrate the application of physico-chemical principles by examples taken from the fields of biology, physiology, and medicine. Since the book is intended chiefly for students of these sciences, the use of mathematics has been avoided as far as possible, and the reader is assumed to have only an ordinary acquaintance with physics and chemistry. Numerous refer-

ences to original papers are given for the use of those who may desire to follow up any particular line.

Of larger works which have been frequently consulted in the preparation of this volume, the following may be mentioned: *Physikalische Chemie der Zelle und der Gewebe*, by R. Höber; *Physikalische Chemie und Medizin*, by Korányi and Richter; *Grundriss der Kolloid-chemie*, by Wo. Ostwald. I desire to thank Professor Benjamin Moore for permission to reproduce Fig. 22, and Herr W. Engelmann for permission to reproduce Figs. 4, 6, 10, and 14. I must also express my indebtedness to Professor Groom, Dr. Harden, and Dr. Senter, who very kindly read parts of the MS. or proofs, and made many valuable suggestions.

J. C. P.

LONDON, *June* 1910.

PREFACE TO THE SECOND EDITION

THE chief new feature of this edition is the addition of a chapter on electromotive force. The treatment of permeability in Chapter V has been extended by a short account of Czapek's researches on the surface tension of the cell membrane, while references to recent work have been inserted throughout.

J. C. P.

LONDON, *October* 1913.

CONTENTS

PHYSICAL CHEMISTRY

CHAPTER I

GASES FROM THE STANDPOINT OF EXPERIMENT AND THEORY. DIFFUSION PHENOMENA

THE recent development of physical chemistry has been characterised very specially by the elaboration and application of new methods for the study of the phenomena exhibited by solutions. These methods are closely related to a theory of solution, which in its turn is rooted in the analogy existing between the behaviour of solutions and that of gases. Any attempt, therefore, to expound the properties of solutions, and the interpretations of these properties that have been proposed, must be preceded by a glance at certain features which characterise the gaseous state, and at various theories which bear on the nature of gases.

Fundamental Experimental Laws.—For physico-chemical purposes the fundamental facts in which the behaviour of gases is revealed are summed up in three laws, two of which are purely physical in character, while the third is more accurately described as chemical. It is perhaps necessary to emphasise the fact that these three laws are absolutely independent of any theories regarding the nature of gases; they are simply the integrated expression of experimental results.

(*a*) *Boyle's Law.*—According to this law, the volume

occupied by a given mass of gas varies inversely as the pressure to which it is subjected, provided the temperature is kept constant. The algebraic expression of this relationship is $pv=C$, where p is the pressure, v is the volume, and C is a constant. This is the equation to a hyperbola, and the curve therefore representing the corresponding changes of pressure and volume for a given quantity of a gas at constant temperature belongs to this type. If we represent by v_1 the volume occupied by a given quantity of gas under the pressure p_1, and by v_2 its volume at the same temperature under the pressure p_2, then we may express Boyle's law by the formula $p_1v_1=p_2v_2$. As an instance of the extent to which this law has been verified in work of the most accurate kind, Lord Rayleigh's figures for hydrogen, oxygen, and carbon dioxide may be quoted.[1] He has shown that if the value of pv obtained for $p=1$ atmosphere is taken as unity, then the value of pv for $p=0{\cdot}5$ atmosphere, instead of 1·0000, is found to be 0·99974 in the case of hydrogen, 1·00038 in the case of oxygen, and 1·00279 in the case of carbon dioxide. For ordinary gases and under ordinary conditions, therefore, Boyle's law may be taken as an accurate statement of the corresponding variation of pressure and volume.

Under certain conditions, however, the formula $pv=C$ ceases to represent accurately the behaviour of a gas at constant temperature, and hence it must be regarded as of only limited validity. Such deviations from strict obedience to Boyle's law occur (1) when the pressure applied to the gas is very high, and (2) when the gas is near its point of condensation. Amagat, for instance, in his experiments[2] on the compressibility of nitrogen at 22°, found that if the value of pv for $p=1$ atmosphere

[1] See *Phil. Trans.*, A, 1905, 204, 351.

[2] *Ann. chim. phys.* (5), 1880, 19, 345.

is taken as unity, the value for $p=62$ atmospheres is as low as 0·986, while for $p=373·3$ atmospheres it is as high as 1·207. Such deviations from Boyle's law, however, become in all cases less marked at higher temperatures.

(*b*) *Gay-Lussac's Law.*—The volume occupied by a given mass of gas, kept under constant pressure, increases as its temperature is raised, and the relative expansion is approximately the same for all gases. It is found that for 1° C. rise of temperature the volume of a gas increases by $\frac{1}{273}$ of the volume which it occupies at 0° C., subject to the condition that the pressure remains the same during the rise of temperature. If v_0 and v are the volumes occupied by a given mass of gas at 0° C. and t° C. respectively under equal pressures, and if $a=\frac{1}{273}$ represents the coefficient of expansion common to all gases, then Gay-Lussac's law may be expressed algebraically by the equation $v=v_0(1+at)$.

From the foregoing, taken in conjunction with Boyle's law, it is easy to show that the pressure exerted by a given mass of gas kept at constant volume must increase with rising temperature in the same proportion as the volume increases at constant pressure; so that, if p_0 and p represent the pressure at 0° C. and t° C. respectively, $p=p_0(1+at)$, subject to the condition that the volume of the gas remains the same throughout. This formula is not only deducible from the equations $pv=C$ and $v=v_0(1+at)$, but is also in harmony with the results of experimental work.

The formula $pv=C$ is applicable only when the temperature is constant, and the formula $v=v_0(1+at)$ only when the pressure is constant; but since the final condition of a gas may differ from its initial condition in respect both of temperature and of pressure, it is obvious that there must be also some one equation connecting the

initial and the final volumes in this case. This equation may be deduced easily in the following manner. We may suppose that a given quantity of a gas, occupying the volume v_0 at the pressure p_0 and the temperature 0° C., occupies the volume v at the pressure p and the temperature t° C.; the problem, then, is to find the algebraic relationship between v and v_0. If we start with the gas at 0° and raise its temperature to t°, the pressure being kept constant, the new volume v_1 occupied by the gas can be easily calculated, for these simultaneous changes of volume and temperature are subject to Gay-Lussac's law; that is, $v_1 = v_0(1+at)$. If now, at the temperature t°, we alter the pressure from p_0 to p, the volume changes from v_1 to v, which we have chosen to represent the final volume. To simultaneous changes of pressure and volume at constant temperature Boyle's law is applicable, so that we have $pv = p_0v_1 = p_0v_0(1+at)$. This equation, $pv = p_0v_0(1+at)$, is the algebraical expression both of Boyle's law and of Gay-Lussac's law. Its validity is not absolute, inasmuch as gases exhibit deviations from strict obedience to Gay-Lussac's law as well as Boyle's law.

If the numerical value of the coefficient a is inserted in the equation it assumes the form $pv = p_0v_0\frac{273+t}{273}$; if, further, it is agreed to reckon temperature, not from 0° C., but from a point 273 degrees lower, and to take T as the symbol of temperature on this new scale, then the equation may be written $pv = \frac{p_0v_0}{273}T$. Temperature reckoned in this way is known as 'absolute' temperature, and -273° C., the starting point of the 'absolute' scale, is termed the 'absolute' zero.

The equation $pv = \frac{p_0v_0}{273}T$ sums up the physical behaviour of gases so far as that is defined by the laws of Boyle and Gay-Lussac, but for certain purposes it is

desirable to have the direct relationship between v_1, the volume which a quantity of gas occupies at pressure p_1 and absolute temperature T_1, and v_2, the volume which it occupies at pressure p_2 and absolute temperature T_2 A little consideration of the foregoing equation will show that the relationship required is $\frac{p_1v_1}{T_1} = \frac{p_2v_2}{T_2}$.

(*c*) *Gay-Lussac's Law of Volumes.*—This third experimental law, although associated with the same name as the second law, is purely chemical in its character, and deals with the relative proportions by volume in which chemical reaction between gases takes place. The law states that when two gases combine with each other to form a third gas, the volumes of the reacting gases are in a simple ratio to one another and to the volume of the gaseous product (all volumes being measured at the same temperature and pressure). Special cases of this will doubtless occur to the reader; it is, for instance, well known that 1 volume of hydrogen combines with 1 volume of chlorine to form 2 volumes of hydrogen chloride, and that 2 volumes of hydrogen combine with 1 volume of oxygen to form 2 volumes of water vapour.

No gas is absolutely 'perfect,' that is, no gas conforms rigidly to the first two laws, and hence it follows that the volume ratio of reacting gases found in the most accurate experimental work is less simple than the preceding paragraph would seem to indicate. At 0° C. and 760 mm., for instance, the ratio of the volumes of hydrogen and oxygen uniting to form water is 2·0028 : 1, according to Scott's accurate investigations.[1] If, however, a correction is made for the slight extent to which hydrogen and oxygen deviate from Boyle's law (see p. 2), the volume ratio is almost exactly 2 : 1.

[1] *Phil. Trans.*, 1893, A, 184, 543. See also Morley, *Zeit. physikal. Chem.*, 1896, 20, 417.

Theories bearing on the Nature of Gases.—(*a*) *The Kinetic Theory of Gases.*—A little consideration of the experimental laws which have just been enunciated shows that gases are characterised by simplicity and uniformity, both in their physical behaviour and in their chemical relationships. Various theories have been brought forward which offer an interpretation of this simple and uniform behaviour. According to one of these, the kinetic gas theory, the ultimate particles of a gas are rushing about at a high speed, the direction of their motion being altered only when they collide with one another or impinge on the walls of the containing vessel. The velocity of motion will vary somewhat from one particle to another, but so long as the temperature of a mass of gas remains the same, the average velocity of the constituent particles will be constant. The pressure exerted by the gas is due to the impacts delivered on the walls of the containing vessel by the moving particles. If it is further assumed that the volume of the particles themselves is negligible compared with the total volume of the gas, and that they are perfectly elastic, it follows by the principles of mechanics that at constant temperature $pv = \frac{1}{3}mnu^2$, in which equation m is the mass of an individual particle of the gas, n is the number of particles in volume v of the gas, and u is the average velocity of the molecules at the temperature considered. So long as the temperature is unchanged the product $\frac{1}{3}mnu^2$ has a constant value, hence $pv = const.$, which is the algebraic expression of Boyle's law. The assumptions of the kinetic gas theory, then, involve the relation between pressure and volume required by the first fundamental law of gases. If the temperature is changed, then the value of pv for a given mass of gas alters according to the formula already discussed—$pv = \frac{p_0 v_0}{273} T$, or,

in words, the product of pressure and volume is proportional to the absolute temperature. But, according to the kinetic theory, $pv = \frac{1}{3}mnu^2$, and, if we are dealing throughout with the same quantity of a given gas, the values of m and n are independent of temperature, so that u^2 must be proportional to the absolute temperature. The kinetic gas theory involves therefore a definite conception of what happens when the temperature of a gas is raised; the kinetic energy of the molecules increases proportionally to the absolute temperature.

(*b*) *Avogadro's Hypothesis.*—According to this hypothesis, which bears especially on the third experimental law, equal volumes of different gases, measured at the same temperature and pressure, contain the same number of ultimate particles or molecules. If it is supposed that when two gases combine chemically one ultimate particle of the first gas reacts with one, two, or three ultimate particles of the second gas, then Avogadro's hypothesis is seen to offer a plausible and natural interpretation of Gay-Lussac's Law of Volumes. But the essential point of the hypothesis, as it was enunciated by Avogadro, lay in the distinction which he drew between atoms and molecules. He suggested that the molecule of an element was not necessarily the same as the atom, and that the ultimate particle of a gaseous element might contain one, two, or more atoms of that element. Only when this suggestion is adopted is Avogadro's hypothesis capable of interpreting all the special cases which are summarised in Gay-Lussac's law.

Avogadro's hypothesis was brought forward primarily as offering an explanation of the volume relationships of chemically reacting gases, but it obviously furnishes also a simple interpretation of the uniform behaviour of different gases when exposed to changes of pressure and temperature. It is noteworthy also that the hypothesis

is found to be in harmony with the consequences of the kinetic gas theory.

The proposition advanced by Avogadro has been adopted as a working hypothesis, and as such has stood the test of time; it is, in fact, a necessary supplement to the Atomic Theory. Since the hypothesis is closely connected with much that is to follow, it is essential to indicate at this stage the results that flow directly from its acceptance.

First of all, the acceptance of Avogadro's hypothesis leads to a definite conception of the relation between the atom and the molecule of the gaseous elements. The argument may be stated as follows. It is a recognised experimental result that 1 volume of hydrogen unites with 1 volume of chlorine to form 2 volumes of hydrogen chloride. If now we suppose that 1 volume of hydrogen contains n ultimate particles of hydrogen, then, according to Avogadro, the 1 volume of chlorine also contains n ultimate particles of chlorine, whilst the 2 volumes of the product contain $2n$ ultimate particles of hydrogen chloride; that is, n ultimate particles of hydrogen unite with n ultimate particles of chlorine to form $2n$ ultimate particles of hydrogen chloride. Since we cannot conceive of an ultimate particle of hydrogen chloride which does not contain at least one atom of hydrogen, the n ultimate particles of hydrogen must have contained at least $2n$ atoms. Hence each ultimate particle, or molecule, of hydrogen must contain *at least* two atoms. There are grounds, which cannot be discussed here, for supposing that the molecule of hydrogen does *not* contain *more* than two atoms; hence we must conclude that the molecule of hydrogen contains two atoms.

The argument may be similarly stated in the case of other gaseous elements.

Secondly, the acceptance of Avogadro's hypothesis

leads to a definite relationship between density and molecular weight. Suppose that D is the density of a gas, and that M is the weight of one molecule: let D_H and M_H represent the corresponding quantities for hydrogen. Suppose also that in unit volume of the gas at N.T.P. (that is, at normal temperature and pressure—0° C. and 1 atmosphere) there are n molecules; then, according to Avogadro, unit volume of hydrogen at N.T.P. also contains n molecules. Now the ratio of the densities of two gases is equal to the ratio of the weights of equal volumes of the two gases, measured of course at the same temperature and pressure; hence $\frac{D}{D_H} = \frac{\text{Weight of unit volume of the gas at N.T.P.}}{\text{Weight of unit volume of hydrogen at N.T.P.}} = \frac{n.M}{n.M_H} = \frac{M}{M_H}$. As has been already shown, the molecule of hydrogen contains two atoms, and therefore the value of M_H is twice the atomic weight. On the basis of O = 16, the atomic weight of hydrogen is 1·008, so that $M_H = 2{\cdot}016$. If, further, it is agreed to refer the density of gases and vapours to that of hydrogen taken as unity, then $D_H = 1$, and we have $M = 2{\cdot}016D$. In words, the molecular weight of a gas is approximately equal to twice its density (relatively to hydrogen).

Thirdly, the adoption of Avogadro's hypothesis permits us to cast the equation $pv = \frac{p_0 v_0}{273} T$ into a more general form, although it should be borne in mind that in so doing we are introducing a hypothetical element into what is otherwise an expression of purely experimental laws. It is obvious that if we take weights of two gases in the ratio of their molecular weights, we are taking an equal number of molecules in the two cases, which means, according to Avogadro's hypothesis, that we are taking equal volumes (measured, naturally, at the same temperature and pressure). Hence, if we are dealing with the molecular weight in grams of any gas, a gram-

molecule, or 'mol' as it is called, the volume occupied at 1 atmosphere and 0° C. should always be the same. This gram-molecular volume must be the volume occupied at 1 atmosphere and 0° C. by 2·016 grams of hydrogen or 32 grams of oxygen, and that has been found to be 22·4 litres.

If, then, it is agreed that in applying the equation $pv=\frac{p_0v_0}{273}T$ the quantity of gas considered is always 1 gram-molecule, the value of v_0 for $p_0=1$ is the same in all cases. Hence the equation may be written $pv=RT$, where $R=\frac{p_0v_0}{273}$ is a constant for all gases. The actual numerical value of R depends on the units in which the pressure and volume are measured; if, for instance, pressure is measured in atmospheres and volume in litres, then $R=\frac{1\times 22\cdot4}{273}=\cdot082$.

The equation $pv=RT$ is termed the gas equation, and is of the utmost importance, not only in connection with gases, but also in relation to the behaviour of dissolved substances, as will appear later. Whenever the equation is applied, it is understood that 1 gram-molecule of the substance is being considered.

Determination of the Molecular Weight of Gases and Vapours.—As has already been pointed out, the acceptance of Avogadro's proposition as a working hypothesis leads to a definite relationship between molecular weight and density, expressed by the equation $M=2\cdot016D$, D being the density of the gas relatively to hydrogen. The determination of molecular weight resolves itself therefore into a determination of density, and it is necessary to consider at least two of the practical methods available for this purpose.

Regnault's Method.—Two spherical glass bulbs, each provided with narrow tube and stopcock, and of approximately equal volume, are required. The one is used merely as a counterpoise on the balance, the other is weighed (*a*) when completely evacuated, (*b*) when filled

at known temperature and pressure with the gas under examination. The volume of the second bulb having been deduced from the quantity of water or mercury

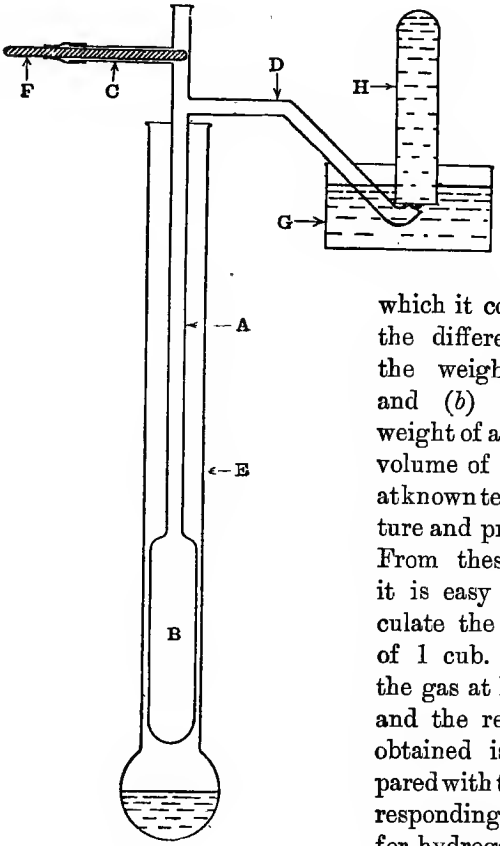

FIG. 1.

which it contains, the difference of the weights (*a*) and (*b*) is the weight of a known volume of the gas at known temperature and pressure. From these data it is easy to calculate the weight of 1 cub. cm. of the gas at N.T.P., and the result so obtained is compared with the corresponding figure for hydrogen.

Victor Meyer's Method.—While Regnault's method is specially applicable to substances which are gases at the ordinary temperature, this second method is employed in the case of

substances which are normally liquid, but can be vaporised without difficulty.

The necessary apparatus is sketched in the preceding diagram (Fig. 1). The glass tube A with cylindrical bulb B and the two side tubes C and D is suspended in the wide vessel E, so that the end of the bulb B is a short distance above the surface of the liquid which occupies the bottom of E. The glass rod F, which is pushed through the side tube C, is kept in position by indiarubber tubing; the elasticity of the latter allows F to be temporarily pulled clear of A without any disturbance of its permanent position. The end of the other side tube D is placed under a graduated tube full of water standing in the vessel G. When the apparatus has been set up the liquid in E is boiled and the rate of ebullition is adjusted, so that ultimately the greater part of the tube E is filled with vapour; the liquid in E must have a boiling point at least 20° above that of the liquid the vapour density of which is being determined. The boiling of the liquid in E will obviously expel a certain amount of air from the bulb B and the tube A, but ultimately, when the ebullition is steady, the expulsion of air will cease, and there will be temperature equilibrium between the inner vessel and the surrounding vapour. This state of equilibrium has been reached when after the insertion of a stopper in the top of the tube A no bubbles of air are expelled through D.

A small bottle containing a weighed quantity of the liquid under examination is then dropped through the top of A on to the end of F, and the stopper is re-inserted. When the rod F is pulled back for a moment the bottle falls to the bottom of B, in which a little glass wool has previously been placed to prevent fracture. At the temperature which prevails in B the liquid is rapidly vaporised and a corresponding quantity of air is expelled

in successive bubbles from the end of D. The air thus collected in H is the exact equivalent of the vapour produced in B, and when no more bubbles are expelled, its volume is found by transferring H to a cylinder full of water and measuring at known temperature and pressure in the usual way. Since this volume of vapour has been produced from the known weight of the liquid taken, the weight of 1 cub. cm. of the vapour at N.T.P. may easily be calculated; the density is then obtained by comparing this result with the corresponding figure for hydrogen.

An example may be taken to show how the data obtained in an experiment with Victor Meyer's apparatus are used to calculate the density, and from that the molecular weight. In a particular case 0·1 gram of a substance was weighed out and vaporised in a Victor Meyer apparatus. The expelled air was collected over water and found to measure 32 cub. cm. at 17° C. and 750 mm. pressure. Now the tension of aqueous vapour at 17° is 14·4 mm., and the volume of air expelled, when allowance has been made for this tension, becomes $\frac{32\times273\times(750-14\cdot4)}{290\times760}$ $=29\cdot16$ cub. cm. at N.T.P. This, then, would be the volume of vapour produced if 0·1 gram of the substance were vaporised at N.T.P., and it follows that the weight of 1 cub. cm. of the vapour at N.T.P. would be $\frac{0\cdot1}{29\cdot16}$ gram. The weight of 1 cub. cm. of hydrogen under these conditions is ·00009 gram, and therefore the density of the vapour (relatively to hydrogen) is $\frac{0\cdot1}{29\cdot16\times\cdot00009}=38\cdot1$; the molecular weight is then $38\cdot1\times2\cdot016=76\cdot8$.

Gaseous Diffusion.—One characteristic feature of a gas as compared with a liquid or a solid is its ability to occupy fully any space which is offered to it; it is capable of infinite expansion, and more than that, the occupation of any vacant space by a gas is accomplished with great

rapidity. Again, when two vessels containing two different gases at the same pressure are put into communication with each other, a process of diffusion goes on until the composition of the gaseous mixture is the same at all points. Each gas moves from places where its concentration is high to places where its concentration is low, and equilibrium is not attained until the partial pressure of each gas is the same throughout. Such diffusion or mutual interpenetration is quite distinct from movement of the gas as a whole. A difference of gas pressure in two places may be equalised by mass movement—air currents, for example—but diffusion goes on where such movement is excluded; it is a molecular process.

The kinetic gas theory, which has so far been discussed only in its bearing on Boyle's law and Gay-Lussac's law, supplies a plausible interpretation of these diffusion phenomena. If, as the theory supposes, each molecule is moving at a high speed (a mile per second, more or less, according to the density and the temperature of the gas), it is intelligible that a gas brought in contact with a vacuous space should occupy the latter practically instantaneously. On the other hand, when one gas is diffusing into another gas the rate of advance is naturally much slower, for the forward path of each molecule, on account of the frequent collisions with the molecules of the other gas, is of a zigzag character.

One of the conclusions which can be deduced from the assumptions of the kinetic gas theory is that the velocity with which a gaseous molecule is endowed is inversely proportional to the square root of the density of the gas. It follows from this, for instance, that the hydrogen molecule has a velocity four times as great as that of the oxygen molecule at the same temperature, for oxygen is sixteen times as heavy as hydrogen. If, then, we are correct in suggesting that diffusion pheno-

mena are closely related to molecular velocity, we may expect to find a definite connection between the rate of diffusion and the density of a gas. In circumstances where the molecular movement alone comes into play, the rate of diffusion of a gas ought in fact to be inversely proportional to the square root of its density.

This important relationship was verified by Graham[1] in his experiments on the rate of passage of different gases through minute apertures into a vacuum. The experiments consisted in determining the times required for a given volume of various gases kept at steady pressure to pass through a minute perforation in a metal plate into a receiver which was being constantly evacuated. Graham found that the time required for the escape, or 'effusion' as he called it, of a given volume of any gas was proportional to the square root of its density; in other words, the velocity of effusion of a gas is inversely proportional to the square root of its density.

Some of Graham's results are recorded in the accompanying table. The times necessary for the effusion of a certain volume of gas are given in the second column, while in the third column are the figures calculated on the assumption that the time of effusion is proportional to the square root of the density; in both cases the time required for the effusion of air is taken as unity.

Gas.	Time of Effusion. Experiment.	Theory.
Air	1	1
Nitrogen	0·984	0·986
Oxygen	1·050	1·052
Hydrogen	0·276	0·263
Carbon Dioxide	1·197	1·237

This relationship has been confirmed by Bunsen,[2] who has further based on it a method for the approximate determination of the density of a gas.

[1] Graham, *Chemical and Physical Researches*, p. 95. [2] *Gasometry*, p. 121.

The rate of passage of a gas through a capillary tube into a vacuum is not inversely proportional to the square root of the density; the friction at the interior surface of the tube comes in as a disturbing factor. The velocity of diffusion of one gas into another through a porous diaphragm is inversely proportional to the square root of the density only when the diaphragm is extremely thin.

Static Diffusion of Carbon Dioxide.—Another interesting phenomenon in the same field as the foregoing is the diffusion of a gas through a tube, at one end of which its concentration is kept either at zero or at some constant low value. This case is interesting because of its bearing on the absorption of carbon dioxide at the surface of a leaf.

The gas exchange between the atmosphere and the assimilating cells of a leaf is at one stage simply a process of diffusion through the stomata alone, for Blackman has shown[1] that if these are blocked up no appreciable diffusion of carbon dioxide into the leaf takes place. This being so, the diffusion of carbon dioxide through the stomata must be relatively rapid; indeed, in the case of a certain leaf examined by Brown and Escombe[2] the stomatic openings were found to absorb per sq. cm. of their area as much as 7·77 cub. cm. of carbon dioxide per hour, a figure which is about fifty times as great as the absorption per unit area of a freely exposed solution of caustic alkali. The question whether this was possible led Brown and Escombe to study the free diffusion of carbon dioxide through small apertures into cavities with a comparatively large absorbing surface.

These investigators found that if a tall cylinder communicating freely with the atmosphere contains at the

[1] *Phil. Trans.*, B, 1895, 186, 485, 503.
[2] *Ibid.*, B, 1900, 193, 223.

bottom a layer of caustic alkali, there is a regular flow or drift of carbon dioxide down the cylinder. Provided that the air outside the cylinder is of uniform composition, and the air inside is free from convection currents, a static condition of affairs is established analogous to what is observed when one end of a metal bar is kept at a high temperature and the other end at a low temperature. When the steady condition of diffusion has been attained the rate of flow of the carbon dioxide, as deduced from the amount absorbed, is found to be inversely proportional to the length of the diffusion column. This is what might be expected on general grounds, for the gradient of the line joining two points of fixed different altitude diminishes as the distance between the two points increases.

When now a diaphragm with a circular aperture is placed at the free end of the diffusion column, the process of diffusion and absorption is modified in a remarkable manner. As the size of the aperture is diminished, the diffusive flow *per unit area of aperture* increases rapidly, and when the area of the aperture has become small in comparison with the sectional area of the tube, the amount of diffusing gas is proportional to the *diameter* of the aperture, not, as one might expect, to its area. This bare statement of results is illustrated by the following figures from Brown and Escombe's paper:—

Diameter of Aperture in mm.	Carbon Dioxide diffused per Hour in cub. cm.	CO_2 diffused per sq. cm. of Aperture per Hour in cub. cm.	Ratio of Areas of Apertures.	Ratio of Diameters of Apertures.	Ratio of Total CO_2 diffused per Hour.
22·7	·2380	·0588	1·00	1·00	1·00
12·06	·0928	·0812	·28	·53	·39
5·86	·0556	·2074	·066	·25	·23
3·23	·0399	·4855	·023	·14	·16
2·12	·0261	·8253	·008	·093	·10

These figures make it plain that the diffusive flow,

especially in the case of the smaller apertures, is proportional, not to the area of the aperture, but to its diameter. A similar 'diameter law' has been established for the diffusion of water vapour into flasks containing concentrated sulphuric acid as absorbent, and for the evaporation of water through narrow apertures into desiccated air.

Diffusion through a Multi-perforate Diaphragm.—When a diffusion tube, such as that already described, is covered with a diaphragm containing many small apertures, the diffusive flow is checked to a remarkably small extent. In Brown and Escombe's experiments diaphragms were employed containing 100 perforations (0·38 mm. diameter) per sq. cm. of diaphragm surface. Although the area of the apertures was in this case only about one-ninth of the total area of the diaphragm, the amount of diffusion through the perforations was as great as when there was no diaphragm at all. The obstruction, therefore, which is offered to a diffusive flow by a multi-perforate diaphragm may be nil, and is certainly surprisingly small. This striking result is to be referred to the intensification of the diffusive flow which, as shown by the figures already quoted, accompanies the gradual decrease of aperture. Provided that the perforations in a multi-perforate septum are not too close, each aperture acts independently of the others, according to the diameter law.

The surface of a leaf, regarded as a purely physical apparatus for the diffusion of atmospheric carbon dioxide to the assimilating centres, resembles a multi-perforate diaphragm. The amount of carbon dioxide, then, which enters the stomata will (1) depend on the gradient of density, and therefore on the extent to which the carbon dioxide concentration in the respiratory cavity approaches zero, (2) be proportional to the *linear* dimensions of

the stomatic openings. In view of the fact that the stomatic openings are elliptical in shape, the question may be raised, What is the linear dimension of such an aperture? The answer is based on a study of evaporation from circular and elliptical surfaces of equal area, and is to the effect that, so far as diffusion is concerned, an elliptical tube is equivalent to a cylindrical tube having the same area of cross-section. As regards the gradient of density in connection with the absorption of carbon dioxide by a leaf, the conditions will be most favourable when the partial pressure of the atmospheric carbon dioxide at the surface of the leaf is kept constant by a movement of the air. If the leaf is in perfectly still air, there will be a density gradient for the carbon dioxide *outside* the stomatic openings also, and the maximum possible absorption of the gas by the leaf will be somewhat diminished.

From Brown and Escombe's researches on the leaf of *Helianthus annuus*, it appears that the actual intake of carbon dioxide is only a small fraction of the amount which the diffusion mechanism of the leaf surface, regarded as a multi-perforate septum, is able to deliver. It follows that the partial pressure of the carbon dioxide in the respiratory cavity can be only slightly less than in the atmosphere outside. The passage of carbon dioxide from the air to the assimilating cells is probably most retarded at the walls of the latter. In order to penetrate these the gas must pass into solution in the water with which they are charged, and the subsequent process of liquid diffusion is very slow compared with gaseous diffusion.

CHAPTER II

ABSORPTION OF GASES BY LIQUIDS

Solubility and Absorption.—It will be clear from the closing paragraph of the last chapter that in the gas exchange between an organism and the atmosphere there are other factors involved besides gaseous diffusion. The gases, both those which are being absorbed and assimilated and those of which the organism is ridding itself, pass into solution at some stage or other of the exchange, and the facilities for such an exchange will therefore depend on the extent to which the gases are soluble in the solvent fluid.

The power of a liquid to dissolve a gas varies very markedly with the nature of the gas, and the solubility of a given gas in a given liquid depends on the temperature and the pressure at which the absorption takes place. As regards the first of these factors, it is found in almost all cases that the solubility of a gas in a liquid diminishes as the temperature rises. The relationship between the solubility of a gas and the pressure under which the absorption takes place is comparatively simple, and is embodied in Henry's law.

Henry's Law.—According to this law, the quantity of a gas (either weight, or volume at N.T.P.) dissolved by a given volume of a given liquid at a given temperature is directly proportional to the pressure under which the absorption takes place; if, for instance, the

pressure on the gas is doubled, twice as much of it will be forced into solution.

With what accuracy Henry's law represents the facts may be judged from the numbers in the following table, which refers to the solubility of carbon dioxide in water. P is the pressure (in cm. of mercury) under which the absorption takes place, and V is the volume of carbon dioxide (measured at N.T.P.) which is absorbed by 1 cub. cm. of water at 15°; according to Henry's law the ratio $\frac{V}{P}$ should be a constant.

P.	V.	$\frac{V}{P}$.
69·8	0·944	0·0135
128·9	1·865	0·0144
200·2	2·908	0·0145
236·9	3·486	0·0147
273·8	4·003	0·0146
311·0	4·501	0·0145

If we were to plot the weight of gas dissolved against the pressure under which the absorption takes place, then the curve obtained in the case of a gas which strictly obeys Henry's law would be a straight line. Deviation from the law occurs when there is chemical action between the gas and any substance present in the absorbing liquid In such a case the relation between the pressure and the quantity of gas absorbed is not a linear one. If, then, the study of the mutual behaviour of a gas and a liquid shows that the quantity of gas absorbed by the liquid is not a linear function of the pressure, it may safely be concluded that the gas is entering into chemical union with some constituent of the liquid. An instance of this will be quoted later on.

The definite relationship between a gas and an absorbent liquid is frequently expressed by means of the 'absorption coefficient.' This is defined as the volume

of the gas (reduced to N.T.P.) which is absorbed by unit volume of the liquid under normal pressure (*i.e.* 1 atmosphere). The statement, for instance, that the absorption coefficient of oxygen in water at 20° is 0·031, means that 1 cub. cm. of water at 20° absorbs under 1 atmosphere pressure 0·031 cub. cm. of oxygen (measured at N.T.P.). The following table shows the values of the absorption coefficient for some common gases in water :—

Temperature.	Oxygen.	Nitrogen.	Carbon Dioxide.
0°	·0489	·0239	1·713
10°	·0380	·0196	1·194
20°	·0310	·0164	0·878
30°	·0262	·0138	0·665
40°	·0231	·0118	0·530

The figures quoted show that oxygen is more soluble in water than nitrogen, that carbon dioxide is much more soluble than either oxygen or nitrogen, and that in all cases the solubility diminishes as the temperature rises.

Sometimes the relationship between a gas and an absorbent liquid is expressed, not by the absorption coefficient, but by the 'solubility,' defined as the volume of gas (measured at $t°$ the temperature of experiment) which is absorbed by unit volume of the liquid under any pressure. If A represents the absorption coefficient and l the solubility, the relation between them is given by the equation $l = A(1 + at)$.

Diffusion of a Gas through a Liquid Film.—The velocity of diffusion of a gas through a very thin porous septum is closely related, as we have already seen, to the density of the gas. But a new factor has to be taken into account when we are dealing with the passage or diffusion of a gas across a liquid film. The velocity of this diffusion depends on the power of the liquid to dissolve the gas, and is, as a matter of fact, directly proportional to the absorption coefficient of the gas in the

liquid. The direction of diffusion in such a case is naturally from the side of the film where the pressure of the gas is high to the side where it is low, the gas being taken into solution at the one surface and passed out of solution at the other. Other things being equal, the amount of gas diffusing across such a film in a given time will be proportional to the difference in the pressure of the gas on the two sides.

That the solubility of a gas is all-important in determining the velocity of its diffusion across a liquid film has been shown by Exner[1] for soap bubbles, and by Wiesner and Molisch[2] for vegetable membranes impregnated with water. In both these cases carbon dioxide, although twenty-two times heavier than hydrogen, diffuses much more rapidly than the latter, for the absorption coefficient of hydrogen is small, and of the same order of magnitude as those of oxygen and nitrogen quoted above. Similarly, carbon dioxide diffuses through moist vegetable membranes much more rapidly than oxygen, a fact which is of importance in relation to the gas exchange between the plant and the atmosphere. It should be noted that the presence of water is essential to the diffusion, for the air-dried membranes are almost, if not altogether, impermeable to these gases.[3]

The difference between an easily soluble and a sparingly soluble gas in connection with diffusion across a film of water is easily demonstrated. For this purpose the apparatus shown in Fig. 2 may be employed. The one end of a short, wide tube *a* is opened out slightly, and a piece of pig's bladder is tied over it and well sealed. The other end is closed by a rubber stopper

[1] *Sitzungsber. k. Akad. Wiss. Wien*, 1874, **70**, ii. 465.

[2] *Ibid.*, 1889, 98, i. 670.

[3] Wiesner and Molisch, *loc. cit.*; see also Steinbrinck, *Ber. deutsch. Bot. Ges.*, 1900, **18**, 275.

carrying a tube *b*, which in its turn is connected with some arrangement for indicating changes of pressure.

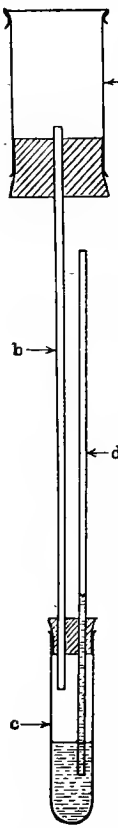

FIG. 2.

In the apparatus sketched in Fig. 2, any increase of pressure in *a* is transmitted to the surface of the coloured liquid in *c*, and the liquid is forced up the tube *d*. If now, when the membrane has been impregnated with water and the apparatus has then been set up, a beaker is inverted over *a* and filled with hydrogen, no appreciable movement of the liquid in *d* is observed. A positive result, however, is obtained when the hydrogen in the beaker is replaced by a gas which is very soluble in water; with ammonia, for instance, a distinct rise of the liquid in *d* is observed in a very short time.

The power of water to dissolve oxygen and carbon dioxide is an all-important fact in connection with aquatic plants. The possibility of gaseous interchange between the air and the cells of the submerged plant depends in the first place on the diffusion of oxygen and carbon dioxide through the medium surrounding the plant, for if the medium is freed and kept free from air the plant dies. In general the epidermis of the submerged leaf is not cuticularised, and is unprovided with stomata. It is, however, impregnated with water, and the exchange of oxygen and carbon dioxide between the surrounding medium and the interior of the leaf consists in a diffusion across this water-logged layer. It has been shown[1] that this diffusion across

[1] Devaux, *Ann. Sci. Nat.*, 1889, [vii.], 9, 35.

the walls of submerged plants is subject to the same laws as regulate the passage of gases across a film of water. The gaseous interchange of aquatic plants must therefore be a comparatively slow process, but in the characteristic development of intercellular spaces there is a mechanism which deals with this difficulty. By this means the oxygen and carbon dioxide liberated in the processes of assimilation and respiration respectively are kept available for future use. Thus it is that those parts of aquatic plants which lie in the mud at the bottom are supplied with oxygen without depending on the slow diffusion of this gas through the surrounding water.[1]

Another point of interest in connection with the gas exchange of aquatic plants is the fact that marine algæ flourish more luxuriantly in arctic than in tropical waters. This is due to the greater solubility of carbon dioxide in water at low temperatures and the resulting increase in the facilities for gaseous interchange.

Various investigators regard the gas exchange which takes place through the walls of the lungs as determined simply by the principles which govern the diffusion of a gas across a liquid film.[2] It has been estimated that the lung surface, regarded as a purely physical apparatus, would allow the diffusion of about 1450 cub. cm. of oxygen per minute in the case of an adult, and some physiologists hold, therefore, that there is no need to assume the existence of a special secretive power in the lung membrane. This is a point, however, on which there appears to be considerable difference of opinion, many physiologists[3] holding, on the other hand, that the lung membrane is the scene of a special secretive action. They maintain that the pressure of oxygen in the blood

[1] See Goebel, *Pflanzenbiologische Schilderungen*, ii. 252.

[2] A. and M. Krogh, *Skand. Arch. Physiol.*, 1910, 23, 179.

[3] See Bohr, *Nagel's Handbuch der Physiologie*, i. 142; Douglas and Haldane, *Journ. Physiol.*, 1912, 44, 305.

is, under certain circumstances, higher than that in the lung cavities. If this is so, then the actual direction of diffusion is opposed to that which the physical law demands.

The air-bladder of fishes is undoubtedly a case in which we must assume a special secretive activity. By keeping a fish alternately at the surface and at various depths below the surface it is possible to bring about variations in the percentage of oxygen in its air-bladder. This organ is the scene of a secretion of oxygen, and the process is under the control of the nervous system.[1]

Solubility of Gases in Salt Solutions.—As a general rule, a gas is less soluble in a salt solution than it is in pure water at the same temperature, and the more concentrated the salt solution the greater is the lowering of the solubility. It is on an analogous principle that the 'salting out' of organic compounds, sparingly soluble in water, is based. The lower absorptive power of salt solutions may be illustrated by the following figures for the solubility of oxygen at 25° in half-normal, normal, and twice-normal solutions of sulphuric acid and sodium chloride; the solubility of oxygen in pure water at this temperature, it should be noted, is 0·0308:—

	$\frac{N}{2}$.	N.	2N.
Sulphuric acid	0·0288	0·0275	0·0251
Sodium chloride	0·0262	0·0223	0·0158

It is interesting to contrast with this the power of blood to absorb oxygen. Amongst other things blood contains in solution appreciable quantities of salts, and hence, in accordance with the general rule just discussed, one might expect the solvent power of blood for gases to be lower than that of water at the same temperature.

[1] Bohr, *loc. cit.*, 163.

Now at 15° C. and 150 mm. pressure (that is, the partial pressure of oxygen in the air) 100 cub. cm. of water can absorb about 0·7 cub. cm. of oxygen, but 100 cub. cm. of dog's blood absorbs under these conditions about 24 cub. cm. of that gas. If the blood is centrifuged, and the corpuscles are thus separated from the plasma, it can be shown that 100 cub. cm. of the latter take up under the afore-mentioned conditions 0·65 cub. cm. of oxygen. The solvent power of the plasma is therefore slightly less than that of water, and it is evidently the corpuscles which are responsible for the greater absorptive power of blood as a whole compared with water.

This difference between the plasma and the blood as a whole is brought out also by a study of the way in which the quantities of oxygen dissolved in the two media are affected by altering the pressure under which the absorption takes place. In the case of the plasma the quantity of gas dissolved is proportional to the pressure —a behaviour in strict accordance with Henry's law. With the blood as a whole, on the other hand, there is no such proportionality. At low pressures the increase in the amount of gas dissolved for a given rise of pressure is much greater than at high pressures; the quantity of oxygen taken up by the blood at 760 mm. pressure is not very much greater than the quantity absorbed under 150 mm. pressure, although on the basis of Henry's law it ought to be about five times as great. The accompanying figure (Fig. 3) will make clear the essential difference between blood and plasma in relation to oxygen.

As has already been pointed out, deviation from strict adherence to Henry's law means that the gas is entering into combination with the solvent or with something dissolved in the solvent. So it is in this case; the absorption of oxygen by the blood is not merely a

physical process; the gas is chemically fixed by the hæmoglobin in the corpuscles, and as the formation of the compound is tolerably complete at low pressures, the form of the upper curve becomes intelligible.

The absorption of carbon monoxide and carbon dioxide by the blood is, it should be noted, subject to similar influences.

A brief reference has already been made to the general

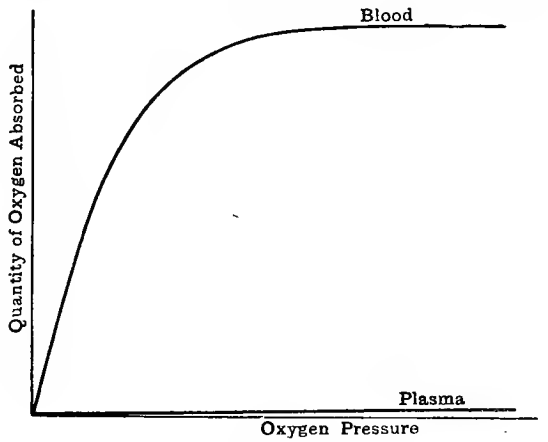

FIG. 3.

rule, that a gas is less soluble in a salt solution than in pure water at the same temperature. In addition to salts, acids, and bases, however, there are other substances, such as sucrose (cane sugar), which have a similar effect in lowering the solubility of gases. The question as to the cause of this influence has lately attracted a good deal of attention, inasmuch as it appears to be closely related to the larger question of the possible hydration of dissolved substances, and therefore also to the important problem of the nature of solution.

One salient fact which has emerged in the study of

the influence of salts on the solubility of gases is, that the relative effects of different salts are nearly independent of the particular gas employed. It has been found that when a number of salts are arranged according to the magnitude of their influence on the solubility of one gas, the order is in general the same as when they are arranged according to the magnitude of their influence on the solubility of another gas. It follows, therefore, that the diminished solvent power of a salt solution as compared with water is mainly determined, not by the specific nature of the dissolved gas, but by some factor which is involved in the relationship of the water and the salt. In support of this conclusion a number of salts, acids, and bases are arranged in the following table[1] according to the magnitude of their influence at the same concentration on the solubility of carbon dioxide (I.), hydrogen (II.), and nitrous oxide (III.). The substances are so arranged that the influence increases from the top to the bottom of the column, and it will be observed that the relative positions are nearly the same in each case.

I.	II.	III.
HNO_3	HNO_3	HNO_3
HCl	HCl	HCl
H_2SO_4	H_2SO_4	H_2SO_4
CsCl	LiCl	CsCl
KNO_3	KNO_3	KNO_3
KI	KCl	KI
RbCl	$NaNO_3$	KBr
KBr	NaCl	LiCl
KCl	KOH	RbCl
NaCl		$NaNO_3$
		KCl
		KOH

Another important experimental result which must be kept in view by any one who attempts an inter-

[1] See Geffcken, *Zeit. physikal. Chem*, 1904, 49, 284.

pretation of these phenomena is, that the influence of a given salt in lowering the solubility is greatest in dilute solution. To extract this result from the actual experimental data, it is necessary to deal with what is known as the 'equivalent relative lowering of the solubility.' Suppose that l_0 is the solubility of a gas in pure water, and that l is its solubility in a salt solution containing n gram equivalents per litre, then $l_0 - l$ is the lowering of solubility and $\frac{l_0 - l}{l_0}$ is the relative lowering of solubility. As a glance at the table on p. 26 will show, the value of $\frac{l_0 - l}{l_0}$ increases as the concentration of the salt solution increases. If, however, we compare the values of $\frac{1}{n} \cdot \frac{l_0 - l}{l_0}$, the equivalent relative lowering of the solubility, that is, if we take the values of the relative lowering per gram equivalent of salt, there is a decrease as the concentration of the salt solution increases. That means, to take a special case, that the efficiency of sodium chloride in lowering the solubility of a gas is in normal solution less than twice as great as the efficiency of this salt in semi-normal solution. The following data, relating to the lowering of the solubility of hydrogen at 15° by sodium chloride and potassium nitrate, will make this point clear; the figures in the table are the values of $\frac{1}{n} \cdot \frac{l_0 - l}{l_0}$.

Concentration of Salt Solution.	Sodium Chloride.	Potassium Nitrate.
1·0 normal	·22	·19
2·0 „	·20	·16
3·0 „	·18	·14

The fact that the value of $\frac{1}{n} \cdot \frac{l_0 - l}{l_0}$ increases with dilution is a result of the utmost importance, for it shows that the cause which is at work in lowering the

solubility is relatively most potent in dilute solutions. It has sometimes been suggested that the influence of salts on the solubility of gases is specially marked in concentrated solution, but the experimental evidence is distinctly opposed to this view.

Interpretations of the Lowering of the Solubility of Gases.—It would be going beyond the scope of this volume to discuss in detail the attempts that have been made to interpret the effect which salts and some non-electrolytes have on the solubility of gases. It is, however, desirable to indicate briefly what explanations have been suggested.

First, it has been maintained that the influence exerted by a salt is connected with the internal pressure of the solution. When a salt is dissolved in water, there is an increase of the internal pressure, which is regarded as equivalent to a corresponding increase of the external pressure. This would mean an extra resistance offered to any increase in the bulk of the liquid, such an increase, for instance, as that which results from the absorption of a gas. Owing, then, to the increased resistance to expansion brought about by the salt, less gas will be absorbed by a salt solution than by pure water.

Some workers who adopt this first line of explanation prefer to deal with compressibility instead of internal pressure, maintaining that the power of a liquid to dissolve a sparingly soluble gas is quantitatively related to its compressibility.[1] Attention is drawn to the parallelism between the lowering of compressibility and the lowering of gas solubility which are the result of adding salts to water.

Secondly, it has been suggested that the lower solvent

[1] Ritzel, *Zeit. physikal. Chem.*, 1907, 60, 319.

power of a salt solution as compared with water is due to the hydration of the dissolved salt.[1] Part of the water in a salt solution is supposed to be in combination with the salt, the solvent which is thus appropriated by the salt being no longer free to absorb gas. The influence of some non-electrolytes[2] in lowering the solubility of gases may be regarded from the same point of view. If it is supposed that the non-electrolyte or electrolyte, as the case may be, is not responsible for any absorption, then the solvent powers of different salt solutions for gases can fairly be compared only when we put side by side the figures for a definite quantity of *solvent* in each case; we must consider the quantity of gas absorbed, not by unit volume of the solution, but by that volume of solution which contains unit volume of the solvent. For it must be borne in mind that in many cases 1000 cub. cm. of a concentrated aqueous solution do not contain anything like 1000 grams of water. A litre of a 10 per cent. sucrose solution, for instance, contains at ordinary temperatures only about 934 grams of water.

On the basis of these assumptions, it is possible to calculate from the lowering of the absorption coefficient the 'average molecular hydration' of a dissolved electrolyte or non-electrolyte, that is, the number of molecules of water which on the average are attached to one molecule of dissolved substance. In the case of sucrose, to take a special instance, the average molecular hydration is about 6, a figure which agrees well with the values obtained by other methods.[3]

[1] Rothmund, *Zeit. physikal. Chem.*, 1900, **33**, 413; Philip, *Trans. Faraday Soc.*, 1907, 3, 140.

[2] That is, substances like sucrose or dextrose, the aqueous solutions of which do not conduct the electric current to any appreciable extent.

[3] See Jones and Getman, *Amer. Chem. Journ.*, 1904, 32, 319; Callendar, *Proc. Roy. Soc.*, A, 1908, 80, 499.

CHAPTER III

OSMOTIC PRESSURE

Diffusion and Osmotic Pressure.—In a previous chapter reference has been made to the diffusion of gases, to the tendency they exhibit to move from places where the concentration is high to places where the concentration is low. Diffusion, however, is a phenomenon which is characteristic not only of gases but also of solutions, and in the latter case also is to be regarded as a molecular movement, not as a movement in mass. If a layer of strong sugar solution is put at the bottom of a tall cylinder, and water is carefully added, with as little mixing as possible, a process of diffusion commences which does not cease until the sugar concentration is the same at all points throughout the liquid. The sugar moves from places where its concentration is high to places where its concentration is low, although naturally, owing to the greater friction, the rate of movement is very much below that observed in gaseous diffusion. In addition to recognising this common characteristic of diffusion we may, in considering the analogy between gases and dissolved substances, go a step further, and regard the movement as due in each case to a pressure. Just as we speak of the pressure of a gas driving the molecules from places of high concentration to places of low concentration, so we may, by way of analogy at least, regard the molecules of a dissolved substance

as diffusing under the influence of a pressure—the *osmotic pressure*, as it is called.

Semi-permeable Membranes.—Gaseous pressure may be realised and measured at some surface interposed to prevent further expansion. Similarly, osmotic pressure might be realised and measured at some surface interposed to prevent further expansion, that is, diffusion, of the dissolved substance. If, however, this surface (some kind of membrane, for instance) is to reveal to us and enable us to measure the tendency to expansion of the dissolved substance *only*, then it must allow free passage to the solvent and block the further advance of the dissolved substance; it must differentiate between solvent and solute; it must be 'semi-permeable.' Given that a solution is separated from the solvent by a surface or membrane satisfying these specified conditions, then diffusion of the dissolved substance is impossible. Such a system, however, is not in equilibrium, for, so long as no hydrostatic pressure develops, equilibrium would be reached only when the concentration of the dissolved substance is the same on both sides of the membrane. Since diffusion of the dissolved substance is barred, the system seeks to get into the condition of equilibrium in the only other way which is possible, namely, by water passing through the membrane into the solution.

This, then, would be the effect of interposing a semi-permeable membrane between solvent and solution, and the next question that arises is, Are such membranes known? The answer is in the affirmative, for certain membranes have been discovered which are readily permeable to water and are found to be practically impermeable to various dissolved substances. There is, for instance, the membrane which is formed when a drop of copper sulphate solution, on the end of a narrow

glass tube, is introduced into a solution of potassium ferrocyanide. At the common surface of the two solutions copper ferrocyanide is deposited as a thin transparent skin surrounding the drop of copper sulphate. Once the skin has been formed the precipitation of copper ferrocyanide ceases, the solutions on either side remaining clear; this obviously means that neither copper sulphate nor potassium ferrocyanide can penetrate a membrane of copper ferrocyanide. This membrane has been found to be impermeable also to various other substances, notably sucrose and dextrose; it may therefore be described as semi-permeable in regard to (1) water and sucrose, and (2) water and dextrose.

When an aqueous solution of sucrose, then, is separated from water by a membrane of copper ferrocyanide, we may hope to observe the passage of water into the solution, which has already been described as an inevitable occurrence in such a system. For the purpose of quantitative measurement, and even for the purpose of qualitative demonstration, the easily ruptured membrane of copper ferrocyanide must be supported on some more or less rigid framework. It may, for instance, be deposited in the walls of a small porous pot of unglazed porcelain. The pot which is to be used for this purpose must be well washed, and its walls must be thoroughly impregnated with water. It is then filled nearly to the top with a dilute solution of copper sulphate (2·5 grams per litre), and allowed to stand for a considerable time in a dilute solution of potassium ferrocyanide (2·1 grams per litre). Under these circumstances the salts diffuse through the walls of the pot, meet in the interior, and deposit a film of copper ferrocyanide. When the formation of the membrane is complete the pot is thoroughly washed and soaked in water; it is then ready for use in a way to be described presently.

There is another method[1] of preparing a membrane of copper ferrocyanide for the purpose of demonstration —a method which is in some ways preferable to the first. One end of a glass tube, 50 mm. long and 10 mm. in diameter, is dipped in 20 per cent. gelatin, to which a little potassium dichromate solution has been added. The gelatin film which is thus formed over the end of the tube becomes insoluble in water if allowed to dry in the light; it may then be soaked in water to remove the potassium dichromate. The glass tube is thus closed at one end by a diaphragm of insoluble gelatin, in which a semi-permeable membrane of copper ferrocyanide may be deposited. A solution of copper sulphate of the strength already specified is put inside the little cell, which is then immersed in potassium ferrocyanide solution. The gelatin diaphragm, which of itself is practically colourless, soon begins to assume a brown colour; this gradually deepens until the formation of the membrane is complete.

The vessel carrying the membrane, either the porous pot or the glass tube with the gelatin diaphragm, is then charged with sugar solution, and a rubber stopper carrying a tube of narrow bore is inserted and made tight with a suitable cement. When the pot or glass tube is immersed in water, the level of the liquid in the narrow tube soon begins to rise slowly, and, if the membrane has been well made, ultimately attains a considerable height. If the membrane were strong enough, and no leaks were sprung, water would continue to pass through the membrane until the hydrostatic pressure of the liquid column balanced the tendency of the water to force its way in. In most cases, however, where no special precautions have been taken, the cell breaks down long before this condition of equilibrium has been reached.

[1] Tammann, *Zeit. physikal. Chem.*, 1892, 10, 700.

The Semi-permeable Covering of Barley Grains.—In addition to copper ferrocyanide and other similar precipitation membranes, there are numerous plant and animal membranes which are permeable to water but impermeable to many dissolved substances; they are therefore semi-permeable. An interesting case of a semi-permeable membrane in the vegetable world was described lately by Brown.[1] He has shown that certain barley grains (*Hordeum vulgare* var. *cærulescens*) have a covering which exhibits selective action when placed in aqueous solutions of sulphuric acid and various other substances; water is absorbed by the grains, whilst the dissolved substance cannot gain an entrance. That sulphuric acid cannot penetrate the covering of the grain is shown by the fact that a blue pigment which is present in the aleurone cells, and which is turned red by acids, remains unaffected when undamaged barley grains are soaked in sulphuric acid. On the other hand, any grain the covering of which is imperfect or has been purposely perforated, at once begins to exhibit the colour change denoting the access of acid to the interior. Grains which have been exposed to the action of boiling water for thirty minutes, and which, after this treatment, have lost all power of germinating, behave in the same way as untreated grains, so that the semi-permeable character of the covering does not depend on the activity of living protoplasm.

A sugar solution separated from water by a membrane of copper ferrocyanide draws water through the membrane, and similarly the contents of barley grains steeped in pure water attract water (up to about 70 per cent. of their weight) through the semi-permeable covering with which they are surrounded. If it were possible to replace the contents of a barley grain by a solution of sulphuric acid, then on steeping in water the same phenomenon

[1] *Annals Bot*, 1907, 21, 79; also *Proc. Roy. Soc.*, B, 1909, 81, 82.

would be observed as in the case of the actual barley grain—water would enter through the semi-permeable covering. We may therefore regard the barley seed contents and a solution of sulphuric acid as both capable of attracting water across a semi-permeable membrane, and hence the steeping of barley grains in a solution of sulphuric acid results in a competition for water between the seed contents and the sulphuric acid. In this connection the experiments made by Brown with solutions of sodium chloride are interesting. This salt resembles sulphuric acid in being unable to penetrate the covering of the barley grain, and in the competition for water between the seed contents and a solution of sodium chloride the amount of water which the former can attract depends on the concentration of the salt solution. As this is raised, the barley grains absorb less and less water: the amount absorbed from a 2 per cent. salt solution is about 41 per cent. of the weight of the seeds, while from a saturated salt solution it is only about 14 per cent. By way of contrast, it is instructive to find that when barley grains are steeped in a solution of a substance which can penetrate the seed covering (*e.g.* acetic acid or ethyl alcohol) the amount of water absorbed is nearly the same as when they are steeped in pure water. There is in this case no competition for the water.

Another illustration of the comparative impermeability of seed coverings to certain substances is found in the use of copper sulphate as a fungicide. This salt is highly poisonous for the vegetable organism, and yet it is possible to steep wheat in a solution of copper sulphate and so destroy any adherent fungus spores without affecting the vitality of the seed itself.

Pfeffer's Work on Osmotic Pressure.—At the beginning of this chapter osmotic pressure has been referred to as the driving force under the influence of which the

molecules of a dissolved substance diffuse. With the interposition of a semi-permeable membrane between solvent and solution, diffusion of the solute becomes impossible, and the corresponding reverse movement of solvent into solution is observed. The driving force behind this movement is opposite in direction to the osmotic pressure, but is equivalent to it. Hence if we can measure the tendency of water to pass into a solution through a semi-permeable membrane, if, in other words, we can measure the force of the attraction between solvent and solution, we are at the same time determining the osmotic pressure of the solution.

There are various conceptions current with regard to the nature of osmotic pressure. Some regard it as being of kinetic origin, strictly comparable with the pressure exerted by a gas on the walls of the containing vessel; others consider it simply as the expression of the attraction between solvent and solution; and others still believe it to be closely related to surface tension. But whatever views may be held as to the nature of osmotic pressure, there is no doubt that what is determined in actual measurement is the force with which the solvent seeks to enter the solution through a semi-permeable membrane.

The pioneer work in the direct measurement of osmotic pressure was carried out in the seventies of last century by Pfeffer, then professor of botany in Basle. His experimental results form part of the foundation on which the modern theory of solution rests, and demand therefore at least a brief consideration.

Pfeffer measured the pressure developed in an osmotic cell which was charged with a solution and immersed in water. The osmotic cell consisted of a small porous pot with a semi-permeable membrane of copper ferrocyanide deposited close to the inner surface. By means of various connecting pieces the pot communicated with a closed manometer

which served to register the pressure. The relation of these various parts will be clear from Fig. 4. The necessity for using a closed manometer instead of an open one will be apparent when it is pointed out that with an open manometer so much water would enter the cell that the concentration of the solution would be materially altered. The pressure measured in an open manometer, except for a very dilute solution, would be quite different from the osmotic pressure of the solution put into the cell. With a closed manometer, on the other hand, the entrance of a trace of water develops such a pressure in the cell as prevents any more coming in. Pfeffer estimated that in a cell actually used by him, and capable of holding 16 cub. cm., the amount of water that entered the cell while pressure equilibrium was being attained was not more than 0·14 cub. cm. In all his experiments, moreover, the specific gravity of the contents of the cell was determined before and after the measurement of the pressure, so that, if necessary, allowance could be made for change in concentration.

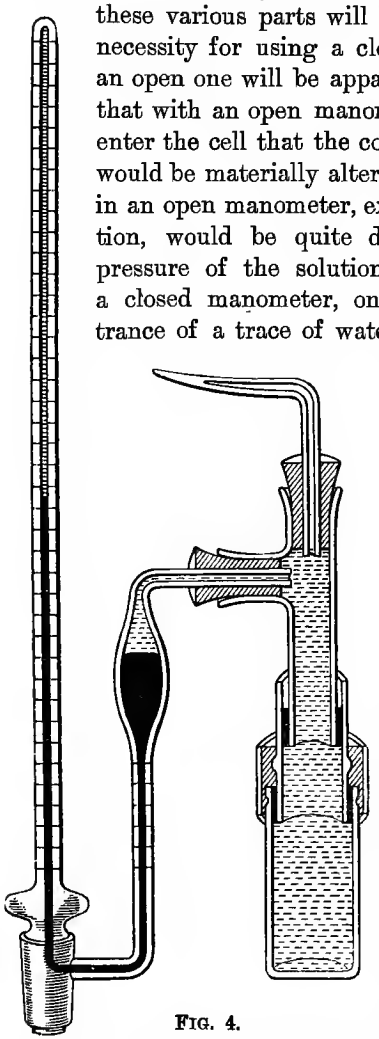

FIG. 4.

With the apparatus just described Pfeffer measured the osmotic pressure of numerous solutions. Evidence of the different osmotic

power of different substances is found in his figures for the osmotic pressure of 1 per cent. solutions of sucrose, potassium nitrate, and potassium sulphate, the values being 53·5, 178·0, and 193·0 cm. of mercury respectively.

It was found also that the osmotic pressure exerted by a given substance in solution increased with the concentration, as shown for sucrose in the first two columns of the following table. The concentration (c) is stated as percentage of sucrose, and the osmotic pressure (P) is given in cm. of mercury. The figures in the third column are the values of the ratio $\frac{\text{pressure}}{\text{concentration}}$, and inspection shows that they are approximately constant.

c.	P.	$\frac{P}{c}$.
1	53·5	53·5
2	101·6	50·8
2·74	151·8	55·4
4	208·2	52·1
6	307·5	51·3

The experimental difficulties encountered in the direct determination of osmotic pressure are very great, and if we ascribe to these the variation from constancy of the figures in the third column, we may conclude that the osmotic pressure of a solution is proportional to the concentration of the dissolved substance. Since the concentration of a given quantity of dissolved substance is inversely as the volume which the solution occupies, the foregoing proposition may be stated also in the following way: The osmotic pressure exerted by a given quantity of dissolved substance is inversely proportional to the volume of the solution. It will be observed that this very closely resembles Boyle's law.

Pfeffer studied also the influence of temperature on the osmotic pressure of a given solution, and showed that the pressure increases as the temperature rises. His

results for a 1 per cent. solution of sucrose are shown in the first and second columns of the accompanying table. The figures in the third column are those cal-

	Osmotic Pressure in Atmospheres.	
$t°$ C.	Observed.	Calculated.
6·8	0·664	0·665
13·7	0·691	0·681
15·5	0·684	0·686
22·0	0·721	0·701
32·0	0·716	0·725
36·0	0·746	0·735

culated by the formula $P=P_0(1+at)$, where $P_0=0·649$ and $a=\frac{1}{273}$—calculated, that is, on the assumption that the osmotic pressure of a given solution is proportional to the absolute temperature.

The agreement between the observed and the calculated figures is far from perfect, but the differences are irregular, sometimes positive, sometimes negative. In view of this and of the afore-mentioned experimental difficulties, we may with reservation assent to the proposition that the osmotic pressure of a given solution is proportional to the absolute temperature—a proposition closely corresponding with the statement of Gay-Lussac's law for gases.

Theoretical Value of the Osmotic Pressure.—Much of the interest attaching to the subject of osmotic pressure is due to the remarkable parallelism between the properties of gases and those of dissolved substances—a parallelism which is revealed in Pfeffer's experimental data, and which was emphasised by van't Hoff in 1887. By a thermodynamical argument,[1] based on the validity of Henry's law, this chemist reached the conclusion that the osmotic pressure of a dilute solution must be proportional (1) to the concentration of the solute, and (2) to

[1] *Phil. Mag.*, 1888, 26, 81.

the absolute temperature. When these propositions were advanced the experimental material available for their verification was very scanty, and was, in fact, derived almost exclusively from Pfeffer's measurements. Nevertheless, van't Hoff was satisfied that his theoretical conclusions were amply verified, and that he was justified in extending Boyle's law and Gay-Lussac's law to solutions.

Moreover, a further step was taken in the extension of Avogadro's hypothesis to solutions, and van't Hoff assumed that at a given temperature equal volumes of two dilute solutions which have equal osmotic pressures contain the same number of dissolved molecules. If this assumption is adopted as a working hypothesis, then it follows that the behaviour of substances in dilute solution must be governed by an equation $PV = R'T$, exactly analogous to the gas equation $pv = RT$. In the equation $PV = R'T$, P is the osmotic pressure, V is the volume of solution which contains 1 gram-molecule of solute, T is the absolute temperature, and R' is a constant for all dissolved substances.

The gas constant R has already been evaluated and found to be 0·082 when the pressure is measured in atmospheres and the volume in litres. Suppose now that we find the value of R', using Pfeffer's data. According to these, the osmotic pressure of a 1 per cent. sucrose solution at 0° C. is 0·649 of an atmosphere; hence $P = 0{\cdot}649$ and $T = 273$. Since we are dealing with a 1 per cent. solution we may, with close approximation to the truth, regard 100 cub. cm. of the solution as containing 1 gram of sucrose; V, the volume of solution which contains 1 gram-molecule of sucrose, that is, 342 grams, would then be 34200 cub. cm., or 34·2 litres. It follows that $R' = \frac{PV}{T} = \frac{0{\cdot}649 \times 34{\cdot}2}{273} = 0{\cdot}0813$, a value very nearly equal to that of R, the gas constant. It appears

therefore that $R' = R$, and that we may employ the same equation to represent the behaviour of dissolved substances as is used in the case of gases, except that what is gas pressure in the one case is osmotic pressure in the other, and that what is the volume occupied by 1 gram-molecule of gas in the one case becomes in the other the volume of solution which contains 1 gram-molecule of solute.

As was pointed out by van't Hoff, the equality of R' and R leads directly to a conclusion of the utmost importance, namely, that the *osmotic pressure of a dilute sugar solution is equal to the pressure which the sugar would exert if it were in the gaseous state at the same temperature, and occupied the same volume as the solution.* Two remarks may be made in reference to this proposition. Firstly, validity is claimed for it only in connection with *dilute* solutions. Van't Hoff himself applied his argument to 'ideal' solutions only, that is, solutions so dilute that the mutual action of the dissolved molecules and their actual volume compared with that of the space they inhabit are negligibly small. Secondly, it does not follow from van't Hoff's proposition that osmotic pressure and gaseous pressure must be due to the same cause; the origin of osmotic pressure may be something quite different from the molecular impacts with which, according to the kinetic theory, the pressure of a gas is associated. Reference has already been made (p. 39) to the various current views of the origin of osmotic pressure, but it ought to be borne in mind that the quantitative relation between the osmotic pressure, temperature, and concentration of a dilute solution is independent of the particular view which may be held in regard to the origin of osmotic effects. In this connection Larmor's views[1] are noteworthy. He supposes that 'each molecule of the dissolved substance

[1] *Nature*, 1897, 55, 545.

sensibly influences the molecules around it so as to form a loosely-connected complex, in the sense, not of chemical union, but of physical influence. Provided the solution is so dilute that each such complex is out of range of the influence of the other complexes, then the principles of thermodynamics necessitate the osmotic laws. It does not matter whether the nucleus of the complex is a single molecule, or a group of molecules, or the entity that is called an ion; the pressure phenomena are determined merely by the number of complexes per unit volume.'

Just as the acceptance of Avogadro's hypothesis leads to a relationship between molecular weight and density, and therefore to a method of determining the molecular weight of gases, so the extension of that hypothesis to solutions may be shown to have a similar result for dissolved substances. The connection between the equation $PV=RT$, in which the hypothesis is incorporated, and the determination of molecular weight is easily traced. For, supposing that a solution of a substance containing g grams per litre is found to have the osmotic pressure P at the absolute temperature T, a simple calculation gives the value of M, the molecular weight of the substance. It must be remembered that V is the volume of solution containing 1 gram-molecule of solute: we have therefore $V=\frac{M}{g}$, and $P\frac{M}{g}=RT$. Since P, g, R, and T are known, M can be calculated.

The determination of osmotic pressure, however, is not a practical method for ascertaining the molecular weight of a dissolved substance, because of the experimental difficulties to which reference has already been made, and which, as experience has shown, are solved only after much patient labour. There are other and simpler methods for determining the molecular weight of dis-

solved substances, methods based on certain properties of solutions that are intimately related to osmotic pressure. These will be discussed later.

Recent Work on the Direct Determination of Osmotic Pressure.—At the time when van't Hoff's theory of osmotic pressure was brought forward, the experimental material available for its verification was exceedingly scanty. Indeed, it is only within recent years that serious and sustained efforts have been made to confirm and extend Pfeffer's work on the direct measurement of osmotic pressure. The result of these efforts has been to throw much light on the relation of osmotic pressure to gas pressure.

Morse, Frazer, and others, in a lengthy investigation,[1] have determined the osmotic pressures of sucrose and dextrose solutions of various concentrations and at various temperatures. The method employed is practically the same as that used by Pfeffer, but the quality of the membranes and the manipulation of the apparatus have been so improved that pressures up to and over 20 atmospheres are regularly recorded with great accuracy. The uncertainties of the measurements are, it is estimated, confined to the second decimal place of the figures expressing the osmotic pressure in atmospheres.

The copper ferrocyanide membranes are deposited electrolytically. The porous pot, thoroughly impregnated with water, is filled with potassium ferrocyanide solution, and placed in copper sulphate solution; a current is then passed from a copper electrode in the copper sulphate to a platinum wire in the ferrocyanide. The copper ferrocyanide is thus deposited in the walls of the pot,

[1] See *Amer. Chem. Journ.*, 1905, 34, 1; 1906, 36, 1, 39; 1907, 37, 324, 425, 558; 38, 175; 1908, 39, 667; 40, 1, 194; 1909, 41, 1, 257; 1911, 45, 91, 237, 383, 517, 554; 1912, 48, 29. See also Professor Findlay's monograph on *Osmotic Pressure.*

the current being continued until the electrical resistance has reached a maximum. The cell is then rinsed out and soaked in water for several hours. This is followed by repeated electrolytic treatment until the resistance ceases to increase. The cell is now filled with a sucrose solution, placed in water, and allowed to develop pressure. When the maximum pressure has been reached the cell is taken down, rinsed, soaked in water, and again subjected to the membrane-forming process. The development of pressure discovers the weak places in the membrane, and these are subsequently mended by the electrolytic treatment. By repetition of these two operations the membrane ultimately reaches a maximum of resistance beyond which it cannot be forced, and the cell gives normal maximum osmotic pressures with sucrose solutions. The membranes prepared in this way are perfectly impermeable to sucrose, even in contact with a solution containing 1 gram-molecule in 1000 grams of water.

Besides effecting this improvement in the quality of the membrane, Morse and his co-workers have perfected (1) the connection between cell and manometer, (2) the means of accurately measuring the pressure. A description of these details, however, lies beyond the scope of this volume.

In considering the results of this recent work on osmotic pressure, we may inquire how far they show that osmotic pressure is proportional to the concentration and to the absolute temperature, how far also they bear out the contention that osmotic pressure and gas pressure are equal. In regard to the first point, it has been found that the osmotic pressure of a sucrose or dextrose solution is proportional to the concentration, *provided the concentration is referred, not to unit volume of the solution, but to unit volume of the solvent.* In the actual work, solutions were made up containing from 0·1 up to 1·0 gram-

molecule of sugar dissolved in each case in 1000 grams of water; these are described as *weight-normal* solutions, in contrast with the *volume-normal* solutions made by dissolving 0·1 or some other fraction of a gram-molecule in water and making the solution up to 1 litre.

The following table will show that the osmotic pressure is proportional to the concentration as just defined; the figures are those for dextrose solutions at 10° C.:—

Concentration in gram-molecules.		Osmotic Pressure.	
Per 1000 gm. of Water.	Per litre of Solution.	In Atmospheres.	Relatively to the first taken as Unity.
0·1	0·099	2·39	1·00
0·2	0·196	4·76	1·99
0·5	0·474	11·91	4·98
1·0	0·901	23·80	9·96

It is obvious that the pressures for the four solutions increase in a ratio which is very nearly that of the concentrations as given in the first column, but diverges widely from the ratio of the concentrations when these are stated in gram-molecules per litre of solution.

As regards the applicability of Gay-Lussac's law to solutions, Morse and his fellow-workers conclude from their numerous experiments between 0° and 25° that the temperature coefficients of osmotic pressure and gas pressure are practically equal. They find, for instance, that the osmotic pressure of a 1·0 weight-normal solution of sucrose is 25·06 atmospheres at 10°, and 26·33 atmospheres at 25°. If the temperature coefficients of osmotic pressure and gas pressure were equal, the osmotic pressure at 25°, calculated from that at 10°, ought to be $\frac{25{\cdot}06 \times 298}{283} = 26{\cdot}38$ atmospheres, in good agreement with the value actually recorded.

The results obtained by Morse and his fellow-workers are of great interest also in relation to van't Hoff's proposition, that the osmotic pressure of a dilute sugar

solution is equal to the pressure which the sugar would exert if it were in the gaseous state at the same temperature and occupied the same volume as the solution. The bearing of the newer experimental data on this proposition will be best appreciated by a study of the following table. It refers to the osmotic pressures of sucrose solutions at 15°, and allows a comparison of the observed osmotic pressures with the values of the gas pressure calculated (I.) on the supposition that the gasified substance occupies the same volume as the *solution*, (II.) on the supposition that it occupies the same volume as the *solvent* in the solution.

Gram-molecules of Sucrose.		Osmotic Pressure in Atmospheres.		
Per 1000 gm. of Water.	Per litre of Solution.	Observed.	Calc. I.	Calc. II.
0·1	0·098	2·48	2·30	2·35
0·2	0·192	4·91	4·51	4·70
0·4	0·369	9·78	8·67	9·40
0·6	0·533	14·86	12·51	14·09
0·8	0·684	20·07	16·07	18·79
1·0	0·825	25·40	19·38	23·49

The values recorded in the last column are much closer to the experimental figures than the values under calc. I., and it follows, therefore, at least for sucrose solutions at 15°, that the osmotic pressure is approximately equal to that which the sucrose would exert if it were gasified at the same temperature, and the volume of the gas were reduced to that of the solvent in the pure state. Similarly, for sucrose solutions at other temperatures between 0° and 25° the observed osmotic pressure is somewhat greater (6–11 per cent.) than the gas pressure at the same temperature calculated on basis II.

The results, then, of Morse and Frazer's work show that Boyle's law is approximately applicable to solutions of sucrose and dextrose up to weight-normal strength,

provided the concentration is referred not to 1 litre of solution, but to 1000 grams of solvent; it has been further shown that from 0° to 25° Gay-Lussac's law applies to solutions, that is, the temperature coefficients of osmotic pressure and gas pressure are equal. The theoretical equality between gas pressure and osmotic pressure is not strictly confirmed in the cases investigated. The excess of osmotic pressure over gas pressure may be due to hydration of the dissolved substance, but the question cannot yet be regarded as settled.

The osmotic pressures of concentrated solutions of sucrose and dextrose have formed the subject of recent investigation also by Lord Berkeley and Mr. Hartley.[1] Except in one or two instances, the solutions examined by these investigators were still more concentrated than those which Morse and Frazer studied. They have also employed another method of determining the pressure which is required to hold in check the tendency of water to enter the solution through a semi-permeable membrane. In Lord Berkeley's experiments the copper ferrocyanide membrane is deposited as near as possible to the outside surface of a porous porcelain tube 15 cm. long, 2 cm. external and 1·2 cm. internal diameter. The means adopted to produce a satisfactory membrane are very similar to those employed by Morse and his fellow-workers. The tube carrying the membrane fits axially into a gun-metal vessel which holds the solution (about 250 cub. cm.); by various devices an absolutely tight joint is secured between the gun-metal vessel and the tube, the open ends of which are exposed. When a determination of osmotic pressure is to be made, a rubber stopper carrying a capillary tube bent at right angles is inserted into each end of the porcelain tube; water is then introduced so as to fill the porcelain

[1] *Phil. Trans.*, A, 1906, 206, 481.

tube completely, and the vertical capillary tubes up to a certain level. The gun-metal vessel surrounding the porcelain tube is now filled up with the solution, and immediately connected with an apparatus by means of which a measured hydrostatic pressure can be applied. It is obvious that if this connection were not made and the solution were left in contact merely with the atmosphere, water would pass from the porcelain tube through the membrane into the solution; this would be accompanied by a fall of the water in the capillary tube.[1] In actual work, however, as soon as the gun-metal vessel is full it is connected with the afore-mentioned apparatus; by means of it pressure is applied to the solution, and so adjusted that water is prevented from entering. If the applied pressure is too great, water is squeezed out of the solution, and this is indicated by a rise of the water in the capillary tube. When the apparatus is so adjusted that the water in the capillary tube remains at a constant level, the registered pressure is the equilibrium pressure of the solution for a pressure of 1 atmosphere on the solvent.

In this way Lord Berkeley and Mr. Hartley have obtained the following values for the osmotic pressures of sucrose and dextrose solutions at 0° C.:—

Sucrose.		Dextrose.	
Grams Sucrose per litre of Solution.	Osmotic Pressure in Atmospheres.	Grams Dextrose per litre of Solution.	Osmotic Pressure in Atmospheres.
180·1	13·95	99·8	13·21
300·2	26·77	199·5	29·17
420·3	43·97	319·2	53·19
540·4	67·51	448·6	87·87
660·5	100·78	548·6	121·18
750·6	133·74		

One of the most remarkable things about these figures is the mere magnitude of the pressures which have been

[1] Only one of the capillary tubes is used for observation; the other is closed by a glass stopcock.

realised and measured. It is indeed a striking result that copper ferrocyanide membranes have been so prepared as to withstand a pressure of 100 atmospheres and over. Even in the cases where the pressure applied to the solution was up to this high figure, only the merest trace of sugar as a rule leaked through the membrane.

A cursory glance at the foregoing tables will show that there is no proportionality between osmotic pressure and concentration, so long, at any rate, as concentration is referred to unit volume of the solution. It is easily seen from a comparison, for instance, of the figures for the first and fourth sucrose solutions and for the first and second dextrose solutions, that the pressure increases much more rapidly than the concentration.

Even when the concentration is referred to 1 litre of the solvent, as was done by Morse and his fellow-workers, the osmotic pressure still increases more rapidly than the concentration. These relationships are best brought out by a diagram, in which the osmotic pressure of sucrose solutions is plotted against the concentration (Fig. 5). Curve I. represents the actual data recorded by Lord Berkeley and Mr. Hartley; curve II. is a straight line, traced on the assumption that the osmotic pressure may be calculated by the equation $PV=RT$, where V is the volume of *solution* containing 1 gram-molecule of sucrose; curve III. is traced on the assumption that the osmotic pressure may be calculated by the equation $PV=RT$, in which V is the volume of *solvent* which goes to 1 gram-molecule of sucrose. The observed osmotic pressure is, it will be seen, always greater than the calculated pressure, even when the latter figure has been obtained by reducing the volume to that of the solvent present. It is, however, obvious that as the solutions become dilute the observed and calculated values approximate more and more closely to each other.

The abnormally high values recorded by Lord Berkeley and Mr. Hartley for the osmotic pressures of sucrose and dextrose solutions have been discussed by Callendar,[1] who finds that the discrepancy between observed and calculated values disappears when it is assumed that the dissolved substance is hydrated. The theory which he develops leads him to conclude that in concentrated

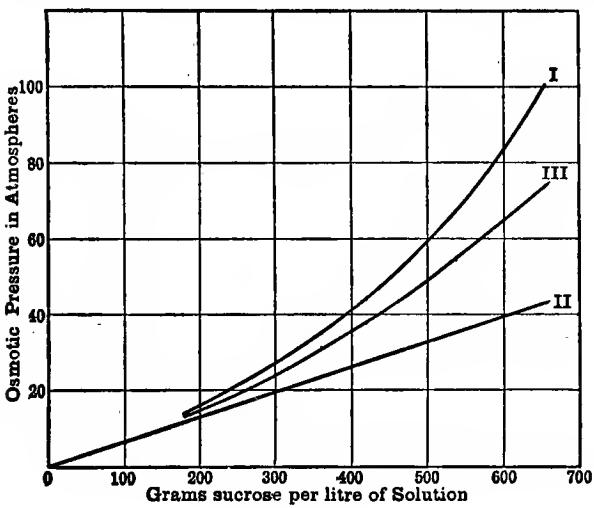

FIG. 5.

sucrose solutions, such as those employed by Lord Berkeley and Mr. Hartley, each molecule of sucrose has attached to it on the average five water molecules. This figure is in good agreement with the value for the average molecular hydration of sucrose deduced from the influence of this substance on the solvent power of water for gases (see p. 32).

[1] *Proc. Roy. Soc.*, A, 1908, 80, 466.

CHAPTER IV

THE COMPARISON OF OSMOTIC PRESSURES. ISOTONIC SOLUTIONS

Water Exchange between Two Solutions of Unequal Osmotic Pressure.—Although the direct determination of osmotic pressure is no easy matter, there are several methods available for the comparison of the osmotic pressures of different solutions. These methods depend on the exchange of water which takes place across a semi-permeable membrane separating two solutions. For two solutions of different osmotic pressure, separated by a semi-permeable membrane, are no more in equilibrium than solvent and solution in the same circumstances. The osmotic exchange of water will always be such as to equalise the pressures on the two sides of the membrane; the water, that is, will pass from the solution with smaller osmotic pressure to the solution which has the greater osmotic pressure.

A simple experiment which demonstrates the existence of this water transport can be made with copper sulphate and potassium ferrocyanide solutions. A tall glass jar is filled with copper sulphate solution of medium strength —say 1 gram-molecule per litre. A little potassium ferrocyanide solution (nearly saturated) is slowly run out from a narrow glass tube, the end of which dips below the surface of the copper sulphate solution. As the potassium ferrocyanide runs out a transparent membrane of copper ferrocyanide is formed where the solutions

meet. A bag containing potassium ferrocyanide solution is thus obtained attached to the end of the tube. When it has become 1–2 cm. in diameter it is detached by jerking the tube, and it then slowly sinks to the bottom of the jar. If the relative concentrations of the copper sulphate and potassium ferrocyanide solutions have been rightly chosen, the latter has not only the greater density but also the higher osmotic pressure. In virtue of this water enters the bag, dilutes its contents, and distends the membrane. The density of the contents of the bag is thereby diminished, and as the water continues to enter, it ultimately becomes equal to and less than the density of the surrounding copper sulphate solution. That this has taken place is shown by the spontaneous ascent of the bag to the top of the jar.

Another experiment which shows even more distinctly the transport of water which takes place across a semi-permeable membrane between two solutions of different osmotic pressure is the following. A small gas jar is half filled with a copper sulphate solution of the same strength as that described in the foregoing experiment. A narrow straight tube, into which some saturated potassium ferrocyanide solution has been sucked up, is closed at the top by a small piece of rubber tubing and a plug of glass rod. The tube is then lowered into the copper sulphate, and the ferrocyanide solution is pushed slightly beyond the end of the tube by compression of the rubber tubing with a screw-clip; a membrane is thus obtained hanging from the end of the tube and separating the concentrated potassium ferrocyanide solution from the weaker copper sulphate solution. In these circumstances water passes from the copper sulphate to the potassium ferrocyanide. One result of this is that the layer of copper sulphate solution which is in immediate contact with the membrane is concentrated, and

becomes denser than the rest of the solution; it therefore sinks, and, on account of the difference in refractive power, the flow or 'trickle' of this denser solution can be readily detected by the naked eye. It is instructive also to make a parallel experiment, in which the ferrocyanide solution is weak and the copper sulphate solution is strong. The ferrocyanide solution is put as before into the tube, which for the purpose of this experiment is bent so that the end immersed in the copper sulphate solution points upward. The water transport in this case is in the opposite direction, from the inside of the membrane to the outside. The copper sulphate solution in immediate contact with the membrane is diluted, and the *ascending* current or 'trickle' of this lighter solution is easily detected.

On these phenomena Tammann[1] has based a method for finding isotonic solutions of two membrane-forming salts—solutions, that is, which have the same osmotic pressure. With the help of an optical apparatus which permits the detection of the slightest irregularity in the refractive power of a medium, it is possible to determine which one of a series of potassium ferrocyanide solutions is isotonic with a given solution of copper sulphate. For when a drop of ferrocyanide solution is introduced into a copper sulphate solution and has surrounded itself with a membrane there is a change of density, and therefore of refractive power, at the top or bottom of the drop, according as the copper sulphate or the potassium ferrocyanide solution has the greater osmotic pressure. Only when the two solutions are isotonic does no irregularity occur in the refractive index either at the top or the bottom. In this way Tammann has found the concentrations of the potassium ferrocyanide solutions which are isotonic with various

[1] *Ann. Physik.*, 1888, 34, 299.

copper sulphate solutions. Some of his results are quoted in the following table, the numbers in which represent gram-molecules per 1000 grams of water. The two figures in the same horizontal line are those of isotonic solutions.

$CuSO_4$.	K_4FeCy_6.
0·84	0·31
0·68	0·24
0·34	0·12
0·20	0·08
0·17	0·066
0·094	0·036
0·049	0·023

The method may be extended to cover other substances than these two salts, so long as they do not pass through a copper ferrocyanide membrane. Suppose, for instance, that a certain solution of potassium ferrocyanide has been found to be isotonic (1) with a solution of copper sulphate, (2) with a solution of copper sulphate + sucrose, it follows that the solutions (1) and (2) must be isotonic. A measure, therefore, of the osmotic pressure of the sucrose in (2) is deducible from the amount of copper sulphate in (1) which it has replaced.

Other methods which are available in comparing the osmotic effects of different substances and in the discovery of isotonic solutions depend on the employment, not of precipitation membranes, but of certain plant and animal membranes. These are permeable to water but impermeable to many dissolved substances, including those in the fluid enclosed by the membrane. Hence, whenever such a membrane with the enclosed fluid is immersed in a solution the osmotic pressure of which differs from that of the fluid, a passage of water occurs in one direction or the other across the membrane.

The Plasmolytic Method.—This method of finding

isotonic solutions of different substances was first employed by the botanist de Vries.[1] It depends on the contraction exhibited by the protoplasm of plant cells when they are immersed in a solution the osmotic pressure of which is greater than that of their own sap. The semi-permeable extensible membrane or bag is the cell protoplasm which encloses the sap and clings closely in the normal state to a surrounding cell wall; this wall is relatively rigid, and is permeable both to water and to dissolved substances. The relation of the protoplasmic membrane enclosing the cell sap to the surrounding cell wall is similar to the relation of a football bladder to the outer leather covering, with this difference, that in the former case any decrease in bulk of the bag containing the cell sap is followed only by a very slight contraction, and still slighter change of shape of the cell wall. Hence if the plant cells under observation have coloured contents any decrease of pressure or of bulk below the normal can readily be detected under the microscope, for the protoplasmic membrane detaches itself at one or more points from the cell wall—a phenomenon which, when brought about by immersion in strong solutions, is known as 'plasmolysis.' The immersion of turgid cells,[2] on the other hand, in a solution which has a lower osmotic pressure than the cell sap produces no visible result, for any water which enters the cell merely increases the pressure inside and pushes the protoplasmic membrane if possible more closely against the supporting cell wall.

The appearance of cells taken from the epidermis of a leaf of *Tradescantia discolor* when they are immersed

[1] *Jahrb. wiss. Botanik*, 1884, 14, 427; *Zeit. physikal. Chem.*, 1888, 2, 415.

[2] Cells, that is, which are in the natural healthy state,—so full that they exert a stretching force on the surrounding cell wall.

in solutions of different strengths is shown is Fig. 6 (de Vries, *loc. cit.*). The shaded parts represent the violet coloured contents of the cells, which are magnified about 300 diameters. In A the normal condition of a cell is represented, as it appears when immersed either in water or in a solution the osmotic pressure of which is lower than that of the cell sap—a 'hypotonic' solution, as is it called. In B the condition of the cell is represented as it appears when immersed in a solution containing

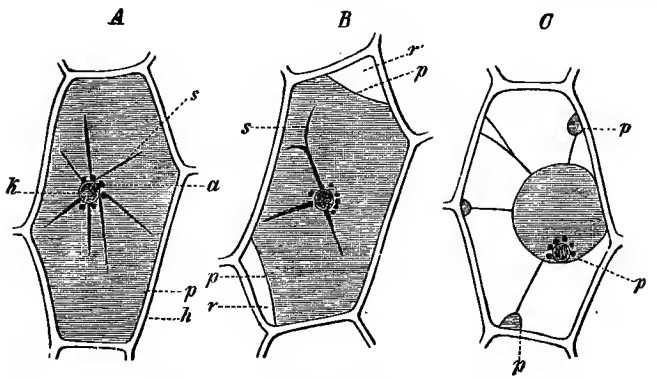

FIG. 6.

k, nucleus; *a*, plastids; *s*, protoplasm stream lines; *p*, protoplast; *h*, cell wall. Magnified 300 diameters.

0·22 of a gram-molecule of sucrose per litre; plasmolysis has taken place, the protoplasmic membrane having drawn away slightly from the cell wall at two points. This solution of sucrose must therefore have an osmotic pressure slightly above that of the cell sap: it is described as a 'hypertonic' solution. In C, finally, there is represented the appearance of a cell when immersed in a solution containing 1 gram-molecule of potassium nitrate per litre; in this case there is very marked plasmolysis; the salt solution is strongly hypertonic.

A ready method of demonstrating the phenomenon of plasmolysis is to make some thin shavings of beet-root, to wash with water in order to remove all juice from the damaged cells, and thereafter to steep for some time in 5 per cent. sodium chloride solution. Examination under the microscope will then show that plasmolysis has taken place. The occurrence of plasmolysis in plant cells with colourless contents is rendered evident by adding to the plasmolysing salt solution some fruit juice or some vegetable dye, such as indigo carmine or aniline blue, which has no injurious effect on the living substance. The salt solution if strong enough produces plasmolysis, the protoplasm retreats from the cell wall at one or more points, and is followed by the dye, which, although it cannot penetrate the protoplasm, can pass through the cell wall.

Isotonic Coefficients.—In order to get isotonic solutions of two salts, it is necessary to find that solution of the one salt which is just strong enough to produce visible plasmolysis in cells of *Tradescantia discolor, Saxifraga sarmentosa, Begonia manicata,* or other suitable plant. The corresponding solution of the second salt, tested by means of cells from the same individual plant, is similarly found, and the two solutions are regarded as isotonic. By way of illustration a special case, investigated by de Vries, may be quoted. He found, using *Tradescantia discolor*, that while no plasmolysis occurred when the cells were immersed in a sucrose solution containing 0·20 of a gm.-mol. per litre, practically all the cells were plasmolysed when treated with a solution containing 0·22 gm.-mol. per litre. Parallel experiments were made with potassium nitrate, and it was found that the two corresponding concentrations in the case of this substance were 0·12 gm.-mol. per

litre and 0·13 gm.-mol. per litre. From these observations the conclusion may be drawn that a sucrose solution containing 0·22 gm.-mol. per litre is isotonic with a potassium nitrate solution containing 0·13 gm.-mol. per litre. The ratio of the isotonic concentrations is in this case 0·13 : 0·22 = 0·59, and it is clear that for equal concentrations potassium nitrate must be osmotically more efficient than sucrose. If we assume with de Vries that the osmotic efficiency increases proportionally to the concentration in each case, then the reciprocal of the foregoing ratio, that is $\frac{1}{0·59} = 1·69$, represents the osmotic efficiency of a potassium nitrate solution when that of a sucrose solution of the same molecular concentration is taken as unity. As standard substance de Vries chose potassium nitrate, the osmotic efficiency of which he took as 3·0. On this basis he found for various substances the following osmotic efficiencies, or 'isotonic coefficients,' as they were called: sucrose 1·81, glycerine 1·78, dextrose 1·88, tartaric acid 2·02, citric acid 2·02, sodium nitrate 3·0, sodium chloride 3·0, potassium acetate 3·0, calcium chloride 4·33, magnesium chloride 4·33, potassium citrate 5·01.

In finding isotonic solutions of two substances by the plasmolytic method the plant cells play merely the part of indicators, and it is not necessary to consider the osmotic pressure of the cell contents. It is, however, pretty evident from the data bearing on the effect of sucrose solutions on the cells of *Tradescantia discolor*, that the cell sap in this case must have an osmotic pressure about equal to that of a sucrose solution containing 0·21 gm.-mol. sucrose per litre. This figure is the mean of 0·20 and 0·22, the concentrations of the highest hypotonic and the lowest hypertonic solutions actually used in the observations. It is not

possible to fix the concentration limits more definitely, for, as de Vries showed, the result of immersing cells of *Tradescantia discolor* in a 0·21 solution of sucrose is that some cells are plasmolysed, others not. It is therefore preferable to take the mean of the highest hypotonic and the lowest hypertonic solution. The osmotic pressure of a sucrose solution containing 0·21 gm.-mol. per litre is, according to Morse and Frazer's investigations, about 5 atmospheres, and this therefore must be approximately the osmotic pressure of the cell sap in the case of *Tradescantia discolor* and many other plants which exhibit plasmolysis in a sucrose solution of about the same strength.

Applications of the Plasmolytic Method.—The extension of Avogadro's hypothesis to solutions is embodied in the statement that equal volumes of two solutions which at the same temperature have equal osmotic pressures contain the same number of dissolved molecules. Hence if we are dealing with two chemically similar substances that may be expected to have approximately the same isotonic coefficient, we conclude that isotonic solutions of these two substances will contain per litre the same fraction of the gram-molecular weight. If the molecular weight of the one substance is known, that of the other may be deduced from it. An interesting application of this is to be found in de Vries' original paper.[1] At that time there was considerable difference of opinion as to the correct formula for crystallised raffinose; as a matter of fact, three formulæ, all consistent with the ascertained percentage composition of the substance, had been proposed, viz. $C_{12}H_{22}O_{11}$, $3H_2O$; $C_{18}H_{32}O_{16}$, $5H_2O$; and $C_{36}H_{64}O_{32}$, $10H_2O$. Now de Vries found

[1] *Zeit. physikal. Chem.*, 1888, 2, 415.

by the plasmolytic method that a 5·96 per cent. solution of raffinose was isotonic with a sucrose solution containing 0·1 gram-molecule per litre. On the basis, therefore, of Avogadro's hypothesis the raffinose solution also must contain 0·1 gram-molecule per litre; that is, the molecular weight of raffinose must be about 596. This result settles the question in favour of the second formula, which requires a molecular weight of 594; the first and third formulæ, on the other hand, would involve molecular weights of 396 and 1188 respectively.

The plasmolytic method was originally employed in the investigation of the pressure which causes turgidity (see p. 58) in plant cells, and since then it has frequently been applied in a similar way. Turgidity is essential to growth, and it is an interesting question what is the pressure prevailing in turgid cells, and what are the means employed to regulate this pressure under varying external conditions. The plasmolytic method furnishes the best means of arriving at a solution of these problems. In order to apply it a substance must first be found for which the protoplasmic membrane of the cells under investigation is impermeable, and then by trial that solution of the substance is found which is just able to produce plasmolysis. The osmotic pressure of this solution gives the pressure prevailing in the cells and producing turgidity, provided that the cell wall was not distended in its normal condition.

How, it may be asked, can it be shown that the plasmatic membrane is impermeable or at least approximately impermeable for any given substance? The guarantee of impermeability in any particular case is found in the observation that plasmolysis when once produced persists even when the cells are left for a considerable time in the plasmolysing solution. If the membrane were slightly permeable to the substance con-

tained in the external hypertonic solution, the plasmolysis would be only transient; the substance would enter the cell, and this would necessarily involve the passage of water in the same direction, leading to the extension of the protoplasmic membrane and the disappearance of plasmolysis. If the membrane were very highly permeable to the substance contained in the external solution, it would be impossible to produce plasmolysis at all. These relationships are well illustrated by the behaviour of certain bacteria,[1] for example *Bacillus cholerœ*, which are temporarily plasmolysed by salt and sucrose solutions, but not at all by glycerine solutions. The plasmolysis observed in the first case disappears in the course of an hour or two as a rule, showing that salt and sugar slowly penetrate the plasmatic membrane. The failure of glycerine to produce plasmolysis points to rapid penetration of the membrane.

Even when a substance has been found for which the membrane is practically impermeable, the result of a plasmolytic experiment must be accepted with some reserve, in so far as the osmotic pressure of the external plasmolysing solution may not be equal to the pressure prevailing in the normal cell. For if the cell wall in its normal state is stretched or distended, then as the osmotic pressure of the external solution approaches that of the cell sap the cell wall must contract; this means that water is squeezed out of the cell, and the sap becomes more concentrated. If we can imagine the osmotic pressure of the external solution being raised gradually, this contraction of the cell wall, and consequent increase in the concentration of the sap, will continue until the cell wall has reached its unstretched condition. Any further increase in the external osmotic pressure beyond this point will produce plasmolysis, but it is obvious that

[1] See Fischer, *Vorlesungen über Bakterien*, p. 20.

the cell sap now in osmotic equilibrium with the external solution is, owing to the contraction of the cell wall, more concentrated than the sap originally filling the cell. In those cases, therefore, where contraction of the cell wall precedes plasmolysis,[1] the plasmolytic values are higher than those corresponding with the original cell sap.

There is another factor which must be taken into account in deciding how far a plasmolytic value gives correctly the osmotic pressure of the cell sap. The cell frequently contains substances which have a feeble osmotic effect, but which readily adsorb water.[2] The total pressure producing turgidity of the cell, as given by the plasmolytic value, may therefore be due partly to the force of imbibition and partly to the osmotic pressure of the cell contents. It is only when the first factor becomes negligible that the plasmolytic value can be regarded as representing correctly the osmotic pressure of the cell contents.

Subject to these reservations, the use of the plasmolytic method has thrown much light on the pressure which prevails in plant cells and on the extent to which this pressure varies from one case to another according to the external conditions. It has been shown,[3] for instance, that the osmotic strength of the cell sap of land plants increases as the acquisition of water becomes more difficult for them. For a bog plant the isotonic solution of sodium chloride was found to contain 0·11 gm.-mol. per litre, for a plant from sandhills 0·24 gm.-mol. per litre, for a plant from the edge of a brackish ditch 0·29 gm.-mol. per litre, and for a salt-marsh plant 0·51 gm.-mol. per litre (= 3 per cent. sodium chloride or 5·2 per cent. potassium nitrate).

[1] See Pantanelli, *Pringsheim's Jahrb. wiss. Bot.*, 1904, 40, 303.

[2] For explanation of adsorption, see Chapter XI.

[3] Drabble, *Biochem. J.*, 1907, 2, 117.

Evidence has, however, been brought forward showing that an individual plant may in certain cases be able to vary its internal osmotic pressure according to that of the surrounding medium. As shown by Hill,[1] this is the case with the root hairs of plants growing in a certain salt marsh, the salinity of which undergoes marked variation owing to periodic inundations by the sea. A sod taken from this salt marsh and containing seedlings of *Salicornia herbacea* was soaked in stream water for eighteen hours. Several seedlings taken from the sod before the soaking were tested, and the root hairs were found to resist plasmolysis in a 5·8 per cent. sodium chloride solution; after the soaking, the root hairs were plasmolysed by a 3·31 per cent. sodium chloride solution. Further experiments showed that the root hairs of *Salicornia* are able also to *raise* their internal osmotic strength in proportion to the increase of the external salinity. The regulation of the osmotic pressure may be effected either by chemical changes in the cell sap or by the passage of sodium chloride through the protoplasmic membrane, but it is uncertain which of these is the mechanism actually employed by the plant.

Very interesting also is the power which some of the lower plants especially have of accommodating themselves to highly concentrated media. This is notably the case with moulds and bacteria; many of these can survive, and even grow in, concentrated salt solutions which would be fatal to the life of the cell in the case of higher plants. *Penicillium* and *Aspergillus* have been found to thrive in solutions the osmotic equivalent of which is 20 per cent. potassium nitrate; *Bacillus anthracis* flourishes on agar containing as much as 8–10 per cent. sodium chloride. Since turgidity is essential to growth, it follows that these organisms must have some means of altering the

[1] *New Phytologist*, 1908, 7, 133.

pressure of their cell contents according to the concentration of the surrounding medium; in this way only can plasmolysis be avoided. As already pointed out, the plasmatic membrane in the case of many bacteria is highly permeable, and it is significant that the permeability is greatest in the case of those bacteria which are best able to thrive in concentrated media. Permeability of the membrane does not however appear to be responsible for the power of accommodation exhibited by moulds; in this case increase in the concentration of the external medium is balanced by the production of osmotically active substances in the cell itself through the agency of metabolic change.[1]

Although some of these lower organisms have such a marked power of accommodating themselves to exceptional osmotic conditions, sudden transference from a very dilute to a very concentrated solution or *vice versa* may have serious consequences for the cell. This is specially obvious in cases where the cell has accommodated itself to a highly concentrated solution of a substance capable of passing only slowly through the membrane; the result of immersing such a cell all at once in pure water is to burst it. If algæ, for instance, are immersed in an isotonic solution of glycerine, and the latter is allowed to evaporate until its concentration rises to about 50 per cent., no plasmolysis will be observed at any time; the glycerine penetrates rapidly enough to prevent this, and the rise of osmotic pressure in the cell keeps pace with the increase of the external concentration. On immersion in water the glycerine begins to pass out, but its escape is far too slow to neutralise the enormous pressure difference between the two sides of the membrane, and the cell therefore bursts. The

[1] Von Mayenburg, *Pringsheim's Jahr. wiss. Bot.*, 1901, 36, 381.

frequent disruption of pollen grains which fall into water is similarly due to osmotic causes.

The value of the plasmolytic method has lately been questioned by Osterhout.[1] In experiments with *Vaucheria* he found that plasmolysis occurred with a sodium chloride solution as dilute as 0·0001N; the addition of calcium chloride however to the sodium chloride solution, although it raises the osmotic pressure, prevents the contraction of the protoplasm from the cell walls. If 1 molecule of calcium chloride is present for every 100 molecules of sodium chloride, the cells may be immersed in 0·1N sodium chloride solutions without suffering plasmolysis. It looks therefore as if in some cases at least sodium chloride exerts a specific effect on the protoplasm, bringing about a contraction which is indistinguishable from plasmolysis caused by purely osmotic action.

Blood Corpuscles and Isotonic Solutions.—The red blood corpuscle is a cell, the contents of which are enclosed in a delicate extensible membrane, permeable to water but impermeable to many dissolved substances. There is, however, no cell wall to give support to the membrane, so that when red blood corpuscles are immersed in water, they first swell up in consequence of the osmotic pressure and then burst. This disruption of the membrane allows the colouring matter, the hæmoglobin, to escape, and the water assumes a deep red colour: the corpuscles are said to be 'laked.' If now a few drops of defibrinated blood are added to each of a series of solutions of sodium chloride of gradually increasing strength, contained in test-tubes, the result obtained for all the solutions up to a certain limiting concentration is similar to that observed in

[1] *Bot. Gazette*, 1908, 46, 53.

the case of water; after sufficient time has been allowed for sedimentation, it is seen that while the bottom of the tube may have a deposit of corpuscles or their transparent envelopes, the supernatant liquid is red. In all the solutions, on the other hand, which are above the limiting concentration, the corpuscles have settled to the bottom, and the supernatant liquid is colourless. Similarly, a limiting concentration may be discovered for another salt, that solution being found by trial which is just dilute enough to lake the corpuscles. The solutions then of the two salts which are equivalent in osmotic effect, as indicated by the incipient laking of the corpuscles, are to be regarded as isotonic solutions.

Hamburger, who is responsible for this method of determining isotonic concentrations, records the following figures,[1] which show how far it is possible in ordinary work to draw a definite line between solutions which lake the corpuscles and those which do not. The figures in Column I. represent the lowest percentage concentration at which the corpuscles sink to the bottom and leave the supernatant liquid absolutely colourless; the figures in Column II. are the highest percentage concentrations at which the corpuscles when they settle leave the supernatant liquid red. Bullock's blood was used in these experiments.

Substance.	I.	II.
KNO_3	1·04	0·96
NaCl	0·60	0·56
K_2SO_4	1·16	1·06
$C_{12}H_{22}O_{11}$	6·29	5·63
CH_3COOK	1·07	1·00
$MgSO_4.7H_2O$	3·52	3·26
$CaCl_2$	0·85	0·79

By careful work it is possible to bring the limits even

[1] *Zeit. physikal. Chem.*, 1890, 6, 319.

closer than is shown in the table, but for ordinary purposes it is sufficient to take as the critical concentration for each substance the mean of the two figures quoted above.

It is perhaps necessary to point out that a 1 per cent. solution of potassium nitrate, which is a critical concentration so far as the corpuscles of bullock's blood are concerned (see above table), is not isotonic with the contents of these corpuscles in their normal condition. If the corpuscles are immersed in a salt solution which is isotonic with their contents, and if the salt solution is then gradually diluted, the corpuscles undergo a corresponding increase in bulk, until at last the limit of resistance of the membrane is reached; the bursting is the final stage in the progressive distension of the corpuscle membrane, and occurs at a concentration considerably below that which is isotonic with the corpuscle contents in their normal condition.

Light is thrown on this question by determining the concentration of the potassium nitrate solution that is isotonic with blood serum. Hamburger showed that a mixture of 10 cub. cm. horse blood serum with 7 cub. cm. of water was unable to lake certain blood corpuscles, although laking took place when 7·5 cub. cm. of water was mixed with 10 cub. cm. of the serum. Corpuscles from the same source were laked by 0·96 per cent. potassium nitrate solution, but not by 0·97 per cent. solution. Hence a mixture of 10 cub. cm. serum + 7·25 cub. cm. of water is isotonic with a 0·965 per cent. solution of potassium nitrate, and we may conclude that the potassium nitrate solution which would be isotonic with the undiluted serum would contain $0{\cdot}965\frac{17{\cdot}25}{10} = 1{\cdot}66$ per cent. of the salt. The corpuscles of horse blood are in osmotic equilibrium with the serum,

so that no great error can be made in regarding the contents of these corpuscles as isotonic with a 1·66 per cent. solution of potassium nitrate. This is quite different from the most concentrated solution of potassium nitrate that is able to lake horse blood corpuscles; that solution contains about 1·17 per cent. of the salt.

It is noteworthy that the limiting concentrations of a salt which produce laking of blood corpuscles are different for different kinds of blood. Thus, for example, the highest concentration of sodium chloride which causes laking is 0·21 per cent. for frog's blood, 0·47 per cent. for human blood, and 0·68 per cent. for horse blood. It is probable that these differences are connected not so much with the varying osmotic strength of the corpuscle contents, as with the different resisting power of the membrane.

Isotonic Solutions found by the Hæmatocrit.—Blood corpuscles may be used in another way for the purpose of finding isotonic solutions. It has already been pointed out that corpuscles immersed in solutions of gradually diminishing concentration increase in bulk, until ultimately the membrane gives way. If, on the other hand, they are immersed successively in solutions of higher and higher concentration, more and more water passes out through the membrane, and the volume of the corpuscles diminishes. It is evident that there must be for each salt which cannot penetrate the membrane some concentration such that corpuscles immersed in the solution undergo no change of volume. This solution is discovered with the aid of the hæmatocrit,[1] a graduated thermometer tube which can be fitted to a centrifuge, and in which the corpuscles collect when blood, either alone or mixed with salt solution, is centrifuged. A

[1] See Hedin, *Zeit. physikal. Chem.*, 1895, 17, 164.

definite quantity of blood, say 10 cub. cm., is treated in this way, and the operation is continued until no further diminution in the volume of the corpuscles in the hæmatocrit can be detected. The same quantity of blood is then mixed with each of a series of salt solutions of graded strength, and the volume of the corpuscles in each mixture is determined as before. That solution in which the volume of the corpuscles is the same as in the blood itself is thus discovered; let it be designated as *A*. Similarly, out of a series of solutions of another salt one *B* is found, in which also the volume of the corpuscles is unaltered. This being so, *A* and *B* are isotonic solutions, and from the isotonic concentrations the isotonic coefficients may be calculated as already shown.

It is interesting to compare the values of the isotonic coefficients obtained by different methods for various substances. This is done in the following table, where the isotonic coefficients are referred to that of sucrose taken as unity :—

	Plasmolytic Method.	Hamburger's Corpuscle Method.	Hæmatocrit Method.
$C_{12}H_{22}O_{11}$ . . .	1·00	1·00	1·00
$MgSO_4$	1·09	1·27	1·10
KNO_3	1·67	1·74	1·84
NaCl	1·69	1·75	1·74
$CH_3.COOK$ . .	1·67	1·66	1·67
$CaCl_2$	2·40	2·36	2·33

The concentration of the sodium chloride solution in which the volume of the corpuscles is unaltered is approximately the same for all mammalian blood, namely, 0·9 per cent. To this solution the term 'physiological salt solution' may properly be applied, for it is the solution in which the corpuscles of mammalian blood remain unaltered as to volume, and in which, therefore, they

may be preserved. The term 'physiological salt solution' is sometimes understood to mean a 0·6–0·7 per cent. sodium chloride solution, but this is a solution in which mammalian blood corpuscles certainly undergo alteration. The lower figure has its origin in the fact that experiments of this kind were first made with frog's blood, the osmotic pressure of which is equal to that of a 0·6 or 0·65 per cent. solution of sodium chloride.

Some Effects produced by Hypertonic Solutions.—The facts discussed in the earlier part of this chapter show that when a plant or animal cell is immersed in a hypertonic solution of a substance which cannot enter the cell, water passes outwards, and the contents of the cell become more concentrated. Such a change of concentration may markedly affect the activity of the cell, as instanced by the following case. The formation of starch from sugar that occurs in many plant cells, takes place only when the concentration of the sugar has reached a certain limit. It is found, however, that even in cells in which the sugar concentration is just short of that limit, starch formation can be induced by plasmolysing with potassium nitrate. The effect of the hypertonic potassium nitrate solution is to raise the concentration of the cell contents beyond the minimum necessary for the production of starch.

Another interesting example of an osmotic stimulus is found in the part which hypertonic solutions play in artificial parthenogenesis.[1] Loeb has shown that when unfertilised eggs of the sea-urchin *Strongylocentrotus purpuratus* are placed for 1½–2 minutes in a mixture of 50 cub. cm. sea-water + 3 cub. cm. 0·1N butyric acid (or other monobasic fatty acid), and are then put back

[1] See Loeb, *Die chemische Entwicklungserregung des tierischen Eies*, 1909; also *Zeit. physikal. Chem.*, 1910, 70, 220.

in ordinary sea-water, a fertilisation membrane is formed in all cases, the appearance of which marks the first stage of development of the eggs. One method of continuing this artificial development is to place the eggs, after the formation of the membrane, in hypertonic sea-water (*e.g.* 50 cub. cm. sea-water + 8 cub. cm. 2·5N NaCl). When they have been allowed to remain 20–50 minutes in this hypertonic sea-water the eggs are placed in normal sea-water, and there develop into larvæ. It is a very interesting fact that a hypertonic sucrose solution may be employed instead of hypertonic sea-water; the result is the same so far as the development of the eggs is concerned. It is not quite certain how the hypertonic solution exerts its influence in this case, but probably it facilitates the oxidation of certain substances which, if not removed, would lead to cytolysis.

CHAPTER V

PERMEABILITY AND IMPERMEABILITY OF MEMBRANES

How does a Semi-permeable Membrane Act?—The consideration of osmotic phenomena leads very obviously to the questions: Wherein lies the efficiency of a semi-permeable membrane? Why is a membrane permeable to one substance, impermeable to another? The answers to these questions have a direct bearing not only on the purely physical side of osmotic pressure, but also on the osmosis which, as indicated in the previous chapter, plays such an important part in the equilibrium between plant and animal cells and their surroundings. The best method, perhaps, of dealing with this problem is first to consider it in its physical aspect alone, and then see how far the information so obtained can help in the interpretation of the biological phenomena of osmosis.

According to Traube,[1] who discovered and studied various precipitation membranes, such as copper ferrocyanide and gelatin-tannin, the feature of a semi-permeable membrane which enables it to differentiate between one substance and another is the size of its molecular interstices. Acting like a sieve, the membrane prevents the passage of particles which have a relatively large volume. Traube indeed maintained that with the help of these precipitation membranes it would

[1] *Archiv. Anat. Physiol.*, 1867, 87.

be possible to estimate the relative size of the particles of dissolved substances.

It is certainly the case that the substances which are suited for quantitative experiments on osmotic pressure, substances therefore which must be practically incapable of penetrating the membrane employed, are all characterised by a high molecular weight. The compounds which have figured in direct determinations are mainly carbohydrates or ferrocyanides, and this fact, if one assumes with Traube that the volume of a molecule depends on its weight and its complexity, seems to support his conception of the action of the membrane.

There are however many other facts which are opposed to this sieve theory of the membrane. In an investigation of the permeability of gelatin-tannin, zinc ferrocyanide, and copper ferrocyanide, Tammann found [1] that of 17 dyes tested 11 penetrated the first membrane, 7 the second, and 5 the third. On the basis of the sieve theory this would mean that the interstices or pores were widest in the gelatin-tannin membrane and narrowest in the copper ferrocyanide membrane. But with individual dyes it was found that in some cases the copper ferrocyanide membrane was more permeable than the zinc ferrocyanide, in other cases the latter was more permeable than the gelatin-tannin membrane, a result quite inconsistent with the sieve theory.[2]

Again, Raoult[3] found that when methyl alcohol and ether are separated by a membrane consisting of pig's bladder, there is an osmotic flow from the alcohol to the ether. If, however, the two liquids are separated by a membrane of vulcanised caoutchouc, osmosis takes place in the opposite direction, that is, from the ether to the alcohol. There must, therefore, be some other

[1] *Zeit. physikal. Chem.*, 1892, 10, 255. [2] See, however, pp. 199, 200.
[3] *Zeit. physikal. Chem.*, 1895, 17, 737.

factor involved besides the size of the pores in the membrane.

The nature of this factor is clearly indicated by Tammann's experiments,[1] which showed that pig's bladder absorbs ten times as much methyl alcohol as ether, and that caoutchouc absorbs about one hundred times as much ether as methyl alcohol. The direction of the osmotic flow is therefore determined by the preferential absorption of one of the two liquids by the membrane. This result was established more definitely by Flusin,[2] who measured the velocity with which water, methyl alcohol, and amyl alcohol pass through pig's bladder, when the other side is bathed by ethyl alcohol, and when the pressure remains equal on the two sides of the membrane. Ethyl alcohol was taken as the second liquid in all cases, because the bladder is practically impermeable to this liquid. Some of Flusin's results are given in the following table; the figures under 'velocity' represent the volume of the liquid in cub. mm. which passed per hour across 1 sq. dcm. of surface, and the figures under 'absorption' are the volumes of each liquid absorbed by 100 grams of bladder in five minutes.

Liquid.	Velocity.	Absorption.
Water	4674	121·9
Methyl alcohol	1748	28·7
Amyl alcohol	646	7·2

The view that the comparative permeability or impermeability of a membrane to different substances depends on its power to dissolve or absorb them to a greater or less extent finds support in the fact that it is possible to construct osmotic cells in which absorption by the membrane is undoubtedly the ruling factor. Reference may be made in this connection to the experi-

[1] *Zeit. physikal. Chem.*, 1897, 22, 490.

[2] *Compt rend.*, 1898, 126, 1497; 1900, 131, 1308.

ment described on p. 24. This experiment, which furnishes an example of gaseous osmosis, showed that in a cell containing air and closed by a membrane impregnated with water, extra pressure is developed when the outside of the membrane is bathed by a gas soluble in water. More definite shape is given to this argument from gaseous osmosis by Sir William Ramsay's work on the pressure produced by the passage of hydrogen through a palladium septum.[1] In these experiments the outside of a small palladium cell containing nitrogen was bathed by a current of hydrogen. The apparatus was kept at 280° by means of a vapour jacket, and the inside of the palladium tube was connected with a manometer to register the pressure. When the initial pressure in the cell was 1 atmosphere, and a current of hydrogen (at atmospheric pressure) had been passed for some time, the internal pressure rose to about 1·9 atmosphere. This increment of pressure in the cell, although just nine-tenths of what might be expected, is plainly connected with the well-known power of palladium to absorb hydrogen, as distinct from other gases; the palladium septum by its absorptive power differentiates between hydrogen and other gases, and so gives rise to osmotic phenomena.

Another osmotic cell, the efficiency of which clearly depends on selective absorption or solution by the membrane, is one described by Crum Brown.[2] Phenol and water are shaken up together until two mutually saturated layers are obtained, namely, (1) a lighter layer containing excess of water, (2) a layer containing excess of phenol. In a portion of the liquid from layer (1) a quantity of nitrate of lime is dissolved sufficient to make the solution heavier than layer (2). This solution

[1] *Phil. Mag.*, 1894, 38, 206.

[2] *Proc. Roy. Soc. Edin.*, 1899, 22, 439.

is then put at the bottom of a narrow cylindrical jar, and above it there is carefully poured a small quantity of layer (2), say about 6–8 mm. in thickness. Above this again is put a considerable quantity of layer (1). The bottom layer in the cylinder is to be regarded as the top layer + calcium nitrate, and the two are separated by a liquid septum in which phenol predominates, and in which calcium nitrate is very sparingly soluble. The medium, however, in which the calcium nitrate is dissolved is readily soluble in the liquid septum, as appears from the fact that phenol and water are appreciably miscible. Hence in the cylinder there is a solution separated from its solvent by a septum which is permeable to the solvent, but nearly impermeable to the dissolved substance. The natural result is that the bulk of the solution gradually increases at the expense of the solvent, and the intervening liquid septum slowly moves up the cylinder. Here again we have a case in which osmosis undoubtedly depends on selective absorption by the membrane.

The physical evidence which has just been quoted gives strong support to the view that the efficiency of a semi-permeable membrane depends on its ability to differentiate, by solvent or absorptive power, between the substances which seek to penetrate it. We may next inquire how far this view can interpret the infinitely more complex phenomena connected with the permeability and impermeability of living membranes. The attempt, however, to give any such interpretation demands first a more detailed discussion of the experimental evidence bearing on the problem, evidence supplied mainly by Overton's work.[1]

[1] *Vierteljahrschrift Zürich*, 1895, 40, 199; 1899, 44, 88; *Zeit. physikal Chem.*, 1897, 21, 189; *Jahrb. wiss. Botanik*, 1899, 34, 669.

Permeability of Living Membranes.—Overton's experiments on the permeability of living membranes were made chiefly with plant cells, but there is remarkable agreement between the behaviour of plant and animal membranes in this respect, and it is true generally that a chemical compound which can penetrate the protoplasm of a plant cell is capable of doing so in the case also of an animal cell. The first method employed by Overton in the systematic study of permeability was the plasmolytic one discussed in the previous chapter, but it is necessary to describe rather more in detail the procedure actually adopted; this is best done by reference to a particular case.

When root hairs of *Hydrocharis* are immersed in a 7·5 per cent. sucrose solution distinct plasmolysis occurs, although none is observed in a 7 per cent. solution. Further, if the 7·5 per cent. solution in which the hairs are immersed is prevented from becoming more concentrated by evaporation, the extent of plasmolysis remains unchanged over a period of twenty-four hours. The plasmolysis vanishes instantaneously when the hairs are dipped in pure water, and reappears with equal readiness when they are replaced in the 7·5 per cent. sucrose solution. The fact that the protoplasmic streaming continues unabated while the hairs are in this solution shows that sucrose exerts no injurious effect on the vitality of the cells. Similar results are obtained with solutions of other substances as well as sucrose, and the conclusion is that the protoplasmic membrane is strictly semi-permeable in these cases. There are however many compounds which, although without deleterious influence on the plant, are unable to produce plasmolysis, or, at the most, produce a temporary plasmolysis. Ethyl alcohol is an example of a chemical compound for which the protoplasmic membrane is highly permeable, and which therefore is

unable to produce plasmolysis. If a *Hydrocharis* root is placed in a solution containing 7 per cent. of sucrose +3 per cent. of ethyl alcohol, no plasmolysis occurs, although this solution is isotonic with a 28 per cent. sucrose solution. The failure of the cells to make any plasmolytic response cannot be due to any injurious influence of the alcohol, for this compound in 3 per cent. solution leaves the majority of plant cells unharmed, except after prolonged contact.

Similar to alcohol in the power of rapid penetration are all monohydric alcohols, aldehydes, ketones, and esters. The dihydric alcohols and the amides of monobasic acids penetrate the cell membrane more slowly, and the permeability is still less for glycerine and urea. In the case of the hexahydric alcohols, the hexoses, the amino-acids, and neutral salts of the organic acids, the permeability is inappreciable.

In some cases another method of studying the permeability of the cell membrane was employed by Overton. The sap of many plant cells contains tannin, a substance which forms sparingly soluble precipitates with numerous chemical compounds; hence when cells containing tannin are dipped in an aqueous solution of one of these compounds, the greater or smaller permeability of the protoplasmic membrane betrays itself by the more or less rapid formation of a precipitate in the cell. In his experiments with caffeine Overton found that the quantity of precipitate formed inside *Spirogyra* cells increased with the concentration of the external solution, while if cells containing precipitate were placed successively in caffeine solutions of gradually decreasing concentration, the precipitate grew less and less. The caffeine, in fact, penetrates the protoplasm with ease, and this is the case also with ammonia, aliphatic amines, and free alkaloids; for the salts of the alkaloids, however, the membrane

is less permeable, and to this fact is probably due the weaker toxic action of these salts compared with that of the free alkaloids.

Another large class of substances, the behaviour of which in relation to the living membrane is interesting, is that of the organic dye-stuffs. Emphasis was laid by Overton on the fact that the salts of the basic aniline dyes, *e.g.* methylene blue, are as a rule very readily taken up by the plant or animal cell, whereas those which are sulphonic acid salts, *e.g.* indigo carmine, either cannot penetrate the cell membrane at all, or do so with great difficulty.

Consideration of all these facts led Overton to the view that, so far as the living membrane can be regarded from the purely physical standpoint, it is selective absorption on the part of the membrane which determines the ability or inability of any substance to enter the cell. The compounds to which the cell membrane is permeable are generally soluble in fatty oils, and it probably consists of a substance which resembles these in solvent power. Overton maintains that the surface layer of the protoplast is impregnated with cholesterol or a mixture of cholesterol with other compounds, such as lecithin, and that the ability of a substance to make its way into the cell depends on its solubility in cholesterol. In support of this contention it has been found that there is a distinct parallelism between the rapidity with which various substances penetrate the cell and the extent of their solubility in cholesterol and lecithin solutions. Overton finds, for instance, that the basic aniline dyes are readily dissolved by solutions of cholesterol and lecithin, but that the sulphonic acid dyes, to which the cell membrane is generally impermeable, are very sparingly soluble in these media.

This theory of the lipoid nature of the plasmatic

membrane has been very widely accepted, but it is not in all respects satisfactory; it fails, for instance, to give a reasonable interpretation of the fundamental fact that the membrane of plant and animal cells is so readily permeable to water. If, in reply to this objection, it is maintained that some of these lipoids are able to take up appreciable quantities of water,[1] one may ask: How is it, then, that simple inorganic salts are unable to penetrate the membrane, or that part of it which is so impregnated with water? In this connection it is noteworthy that Czapek's work on the surface tension of the plant cell (see p. 85*a*) is opposed to the view that the plasmatic membrane is a continuous lipoid film, and rather favours the conception of it as a very fine fat emulsion, permeable for water and substances soluble in water.

The theory has been criticised adversely by Ruhland,[2] who contends that the parallelism between power to penetrate the protoplasmic membrane and solubility in cholesterol solutions is not so complete as Overton believes. There are dyes which are readily soluble in lipoids, and yet are unable, or practically unable, to enter the living cell; while, on the other hand, there are dyes for which the plasmatic membrane is highly permeable, which, however, are almost insoluble in cholesterol. Some workers contend that the plasmatic membrane is protein, rather than lipoid, in character.[3]

The statement that simple inorganic salts are unable to penetrate the protoplasmic membrane is not absolutely correct. The very fact that the cell sap contains salts

[1] Lanolin, for instance, which is obtained from wool oil, and contains appreciable quantities of cholesterol, takes up in the anhydrous state about an equal weight of water.

[2] *Jahrb. wiss. Bot.*, 1908, 46, 1.

[3] See Robertson, *J. Biol. Chem.*, 1908, 4, 1; Osterhout, *The Plant World*, 1913, 16, 129.

which are supplied to the plant in the nutrient medium in which it grows indicates that there must be provision of some kind for the absorption of these salts. Further, a direct proof of the penetration of some inorganic salts into living protoplasm has recently been described by Osterhout.[1] His experiments were carried out chiefly with *Dianthus barbatus*, which can be grown in distilled water, and the root hairs of which during such growth remain free from calcium oxalate crystals. When, however, they are transferred from distilled water to a dilute solution of a calcium salt, the presence of calcium oxalate crystals in the root hairs is evident within a few hours. This shows that calcium salts may penetrate fairly rapidly into living protoplasm. The subsequent growth is normal, so that the penetration of the calcium salts is not due to any abnormal or injured condition of the cells.

Difficulties of a Purely Physical Theory of Permeability.—It must be recognised that the behaviour of the living cell membrane towards the substances with which it comes in contact is in many cases incapable of interpretation on a purely physical basis. Although this is not the place for a discussion of the problem of permeability in its physiological aspect, it is worth while to indicate one or two of the facts in the face of which any purely physical theory is found wanting. It is well known, for instance, that the permeability of a cell membrane alters on the death of the cell; certain dyes can enter the cell only when the latter is killed. Again, there is the very striking fact that the inorganic constituents of the blood corpuscle are notably different from those of the plasma: the corpuscle fluid is comparatively rich in potassium and phosphate, while the plasma is poor in these, but rich in sodium and

[1] *Zeit. physikal. Chem.*, 1910, 70, 408; *Science*, 1911, 34, 187; 1912, 35, 112.

chloride. From the fact that the cell receives its nutriment from the external medium, it appears that the membrane cannot be absolutely impermeable to potassium salts, and yet their retention in the cell would seem to be impossible if permeability of the membrane is conceded. We are therefore driven to assume some specific intervention of the living membrane or some special affinity between the cell protoplasm and the potassium salts.[1]

There are cases also where the membrane surrounding an organ, or even a whole organism, behaves in a way which is incompatible with a purely physical theory of permeability and osmosis. In the processes of secretion it is found that owing to the specific activity of the secretory organs a substance may be transferred from a place where its concentration is low to a place where its concentration is high. In the kidneys, for instance, urea is transferred from the blood, which contains little of it, to the urine, in which the proportion of urea is much greater. This could not be effected by any purely osmotic agency. Reference may be made also to some interesting observations on tadpoles made by Overton. Immersed in a 5–6 per cent. sucrose solution or in a 0·6 per cent. sodium chloride solution, tadpoles are unaffected, and their activity is unimpaired. If they are transferred to an 8 per cent. sucrose solution or an 0·8 per cent. sodium chloride solution, they lose a considerable quantity of water in the course of twenty-four hours, and shrink notably in size. A similar result is produced by immersion of the tadpoles in solutions of uninjurious substances which are hypertonic to a 6 per cent. sucrose solution. Immersion in a solution hypotonic to a 6 per cent. sucrose solution we should expect to be followed by an intake of water, and consequent increase in the size of the tadpoles. This however is not the case, and

[1] See Moore and Roaf, *Biochem. J.*, 1908, 3, 55; 1911, 6, 110.

it therefore appears that the epithelial membranes of the tadpole are permeable to water in the one direction, but not in the other. This fact, and the others which have just been quoted, will serve to show that a purely physical theory of the exchanges which take place across a living membrane is inadequate; there is a physiological permeability as well as a physical permeability.

Permeability and Surface Tension of the Cell Membrane.—As indicated by the phenomena of plasmolysis, the plasmatic membrane of plant cells is normally impermeable to the substances present in the cell sap. There are conditions, however, in which the membrane loses its power of retaining these substances, and the manner in which this may be brought about artificially is of great interest and importance. The researches of Czapek[1] have shown that, as a convenient test for the unimpaired character of the cell membrane, the reaction between tannin and caffeine (cp. p. 81) may be employed. In a very large number of cases tannin is a constituent of the cell sap, and so long as the impermeability of the protoplasmic membrane is intact, immersion of such cells in a dilute caffeine solution will lead to the formation of a precipitate inside the cell. If, on the other hand, the cells have been exposed to such conditions as destroy the impermeability of the membrane, then the tannin will diffuse away, and after a time the reaction with caffeine will be very feeble, if not entirely absent.

In his systematic study of the effect of different substances (in aqueous solution) on the permeability of higher plant cells, Czapek found that for each substance there was a critical concentration, such that the impermeability of the cells was retained if they were immersed

[1] *Ueber eine Methode zur direkten Bestimmung der Oberflächenspannung der Plasmahaut von Pflanzenzellen* (Gustav Fischer; Jena, 1911).

for some time in a weaker solution, but rapidly destroyed if they were immersed in a stronger solution. In the series of the fatty alcohols, for instance, these critical concentrations are about 14 per cent. by weight for methyl alcohol, 8–9 per cent. for ethyl alcohol, 4 per cent. for *n*-propyl alcohol, 1·5 per cent. for *n*-butyl alcohol, and 0·5 per cent. for amyl alcohol.

Now the very significant fact has been established that all these critical solutions - critical, that is, for the impermeability of the cell membrane—have practically the same surface tension, the average value being 0·685 that of water. The conclusion may therefore be drawn that this figure represents the natural surface tension of the plasmatic membrane, and that the abnormal permeability exhibited by higher plant cells after immersion in solutions above the critical concentration, results from the displacement of those substances which are normal constituents of the membrane. Further, the significant observation has been made that the surface tension of the saturated emulsions of neutral fats (containing notably the glycerides of unsaturated fatty acids) has a minimum value of 0·68 relatively to water. The coincidence of this figure with that for the natural surface tension of the plasmatic membrane is suggestive in connection with the question as to the nature of this membrane. Attention may be drawn also to the fact that the power of substances, notably the fatty alcohols, to lower the surface tension of water stands in evident relationship to their physiological activity[1] and to their hæmolytic power.[2]

[1] Traube, *Ber. deutsch. physikal. Ges.*, 1904, 6, 326.

[2] Führer and Neubauer, *Archiv exper. Pathol.*, 1907, 56, 333.

CHAPTER VI

VAPOUR PRESSURE, BOILING POINT AND FREEZING POINT OF SOLUTIONS

Vapour Pressure of Solvent and Solution.—The direct determination of the osmotic pressure of a solution is no easy matter. There are however other properties of solutions which are quantitatively related to osmotic

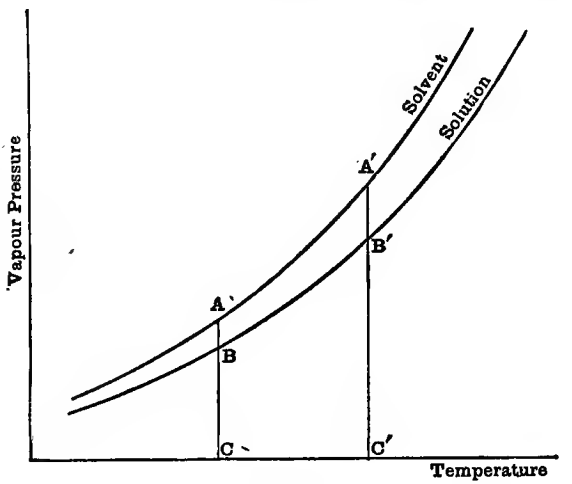

FIG. 7.

pressure, and serve therefore for its indirect evaluation. The first of these is the vapour pressure. Investigation, chiefly by Raoult, has shown that when the dissolved substance is non-volatile the vapour pressure of a dilute

solution is lower than that of the pure solvent at the same temperature by an amount which is proportional to the concentration of the dissolved substance. The general relation between the vapour pressure of the solvent and that of the solution at different temperatures is represented by the curves in Fig. 7, the upper curve showing the variation in the vapour pressure of the solvent with rising temperature, the lower curve showing the corresponding variation in the vapour pressure of the solution. The relative position of the two curves is such that, if AC and BC represent the vapour pressures of solvent and solution at one temperature, A′C′ and B′C′ the same quantities at any other temperature, $\frac{BC}{AC}=\frac{B'C'}{A'C'}$, which means simply that for a given solution the ratio of the vapour pressures of solution and solvent is the same at all temperatures.

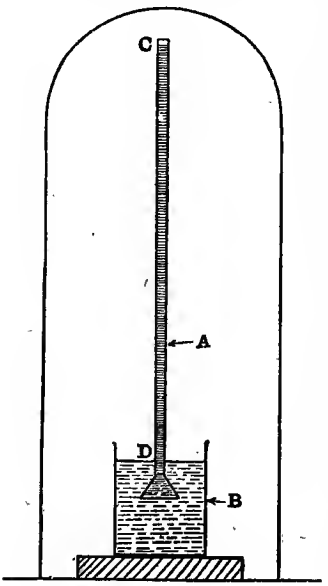

FIG. 8.

Vapour Pressure and Osmotic Pressure. — The mere fact of the existence of osmotic pressure involves the consequence that the solution of a non-volatile substance must have a lower vapour pressure than the solvent at the same temperature. The connection between the two will be made evident by consideration of Fig. 8. Suppose that A is a funnel and tube closed at the bottom by a semi-permeable membrane, and containing sucrose

solution. Suppose also that osmotic equilibrium has been established between the sucrose solution and the pure water in the vessel B, that, in fact, the weight of the column CD is equal to the osmotic pressure of the sucrose solution. If the whole apparatus stands in a vessel from which the air has been removed, the space inside the vessel will be occupied by water vapour. Now in any gaseous atmosphere the density, and therefore the pressure, of the gas is greatest at the bottom, because there the weight of the overlying column is a maximum; the pressure at a higher point is less in proportion as the weight of the gaseous column above is diminished. Hence the pressure of water vapour at C, level with the top of the sucrose solution in A, is less than at D, the surface of the water. The pressure at D is the vapour pressure of water at the temperature of the apparatus, and, since there is equilibrium, the pressure at C must be equal to the vapour pressure of the sucrose solution, the surface of which is at this level; that is, the vapour pressure of a solution must be lower than that of the solvent at the same temperature. Further, it is apparent that the difference between the vapour pressures is equal to the weight of a column of water vapour of height equal to CD.

When the relationship between the osmotic and vapour pressures of a solution is treated mathematically, it is found that $P = \frac{SRT}{M} \cdot \log_e \frac{p}{p'}$, where P is the osmotic pressure and p' the vapour pressure of the solution; p is the vapour pressure, M the molecular weight, and S the specific gravity of the solvent; T is the absolute temperature, and R is the gas constant. A glance at the formula will show that in order to calculate the osmotic pressure of a solution from its vapour pressure it is not necessary to know the absolute values of p and p'; a

knowledge of the *ratio* of the vapour pressure of the solvent to that of the solution is sufficient. It is, as a matter of fact, an easier matter to determine the ratio of the vapour pressures of solvent and solution than to determine their absolute values.

One very simple method of finding the ratio in question when water is the solvent is that devised by Ostwald and Walker.[1] A current of air is drawn slowly through (1) Liebig's bulbs charged with the aqueous solution under examination, (2) another set of bulbs similarly charged, (3) bulbs containing water, (4) a U-tube containing pumice moistened with concentrated sulphuric acid. When the air leaves (2) it is charged with water vapour up to the pressure of the solution; when it leaves (3) it is charged with water vapour up to the pressure of pure water at the same temperature. The air therefore takes up water during its passage through the bulbs (3), and the loss of weight which these bulbs show is proportional to the difference $p-p'$. In passing through the sulphuric acid tube the air is deprived of the whole of the water vapour which it has taken up, and the gain in weight of this tube during an experiment is therefore proportional to p. A determination of the loss in weight of (3), and the gain in weight of (4), after a current of air has been passed for some time, gives the required ratio of the vapour pressures, for $\frac{p-p'}{p} = \frac{\text{loss in weight of water bulbs}}{\text{gain in weight of sulphuric acid tube}}$, and from this $\frac{p}{p'}$ can be easily calculated. Since, as already stated, the value of $\frac{p}{p'}$ is independent of temperature, it is not essential in this method to keep the temperature absolutely constant throughout an experiment; it is necessary only

[1] Walker, *Zeit physikal Chem.*, 1888, 2, 602.

to ensure that the variation of temperature, if any, shall be the same for all parts of the apparatus.

This method of finding the relative vapour pressures of solvent and solution has lately been modified and improved by Lord Berkeley and Mr. Hartley,[1] who arranged that the current of air, instead of bubbling through the solvent and the solution, should pass over their surfaces in specially constructed apparatus; in this way equality of the air pressure is secured throughout the train of vessels. In order to ensure rapid and complete saturation of the air with water vapour the vessels containing the solution and the solvent are regularly rocked, so that the exposed surface of liquid is constantly being renewed. With this apparatus Lord Berkeley and Mr. Hartley have determined the value of $\frac{p}{p'}$ for various solutions of sucrose and calcium ferrocyanide, the osmotic pressures of these solutions being then calculated from the vapour pressure ratio by a formula similar to that quoted above. It is interesting to compare the values thus indirectly obtained for the osmotic pressure of calcium ferrocyanide solutions with those determined by the direct method described on p. 50.

Grams Anhydrous Salt per 100 grams of Water.	Osmotic Pressure in Atmospheres.	
	Observed.	Calculated from Vapour Pressure.
31·39	41·22	41·24
39·50	70·84	70·61
42·89	87·09	86·61
47·22	112·84	112·96
49·97	130·66	131·45

Vapour Pressure and Molecular Weight. — Since there is this quantatitive relationship between vapour pressure and osmotic pressure, and since, as already shown, a knowledge of the osmotic pressure of a solution

[1] *Proc. Roy. Soc.*, 1906, A, 77, 156; *Phil. Trans.*, 1909, A, 209, 177.

permits a calculation of the molecular weight of the dissolved substance, there must be some way of deducing the molecular weight directly from the vapour pressure. The required relationship is given by the formula $\frac{p-p'}{p}=\frac{n}{n+N}$, where p and p' are the vapour pressures, as before, of solvent and solution, n is the number of solute molecules, and N is the number of solvent molcules in the solution. A slight transformation of the formula gives $\frac{p-p'}{p'}=\frac{n}{N}$, so that $n=N\frac{p-p'}{p'}$. In order to illustrate the use of this formula the following data may be considered. A solution of 11·35 grams of oil of turpentine in 100 grams of ether was found to have a vapour pressure of 36·01 cm., the vapour pressure of pure ether at the same temperature being 38·3 cm. The molecular weight of ether is 74, so that the value of N for the given solution is $\frac{100}{74}$, and $n=\frac{100}{74}\times\frac{38{\cdot}3-36{\cdot}01}{36{\cdot}01}={\cdot}086$; that is, 11·35 grams is ·086 of a gram-molecule, and the molecular weight of oil of turpentine is therefore $\frac{11{\cdot}35}{{\cdot}086}=132$, in agreement with the theoretical value 136.

A glance at the formula connecting osmotic pressure and vapour pressure, viz. $P=\frac{SRT}{M}.\log_e\frac{p}{p'}$, shows that when solutions of two non-volatile substances in the same solvent have the same vapour pressure at any temperature, their osmotic pressures must be equal; the solutions are isotonic. Now it has already been shown on p. 62 that if we can find isotonic solutions of two substances, the molecular weight of the one can be deduced from that of the other. Hence it follows that if we can find solutions of two substances which have the same vapour pressure, the molecular weight of the

second can be calculated, when that of the first is taken as known.

An interesting microscopic method of finding isotonic solutions, and therefore also of determining molecular weights, has been described by Barger.[1] During some experiments on the growth of fungi in concentrated solutions it had been noticed that when a drop of strong salt solution is kept for some time in a small enclosed space in which water also is present the size of the drop gradually increases. This is obviously due to the fact that the vapour pressure of the water is greater than that of the salt solution, and hence there results a slow distillation from the water to the solution. In Barger's method a solution B is made up of the substance of unknown molecular weight, as well as a number of solutions A_1, A_2, &c., of a standard substance the molecular weight of which is known; these last solutions form a series of gradually increasing concentration. Alternate drops of A_1 and B are now introduced into a capillary tube, and any variation in the size of the drops is observed under the microscope from time to time. If the size of the B drops increases at the expense of the A_1 drops, it follows that the vapour pressure of B is less than the vapour pressure of A_1, and therefore that the osmotic pressure of B is greater that that of A_1. The experiment is now repeated with the whole series of A solutions, when it will be found that there are two adjoining members of the series A_4 and A_5, we shall suppose, such that when alternate drops of A_4 and B are put in a capillary tube the B drops increase in size at the expense of the A_4 drops, while in a similar experiment with A_5 and B, the A_5 drops increase at the expense of the B drops. The solution of A which has the

[1] *Journ. Chem. Soc.*, 1904, 85, 286.

same vapour pressure and the same osmotic pressure as B must therefore lie between A_4 and A_5. The application of this method may be illustrated by the following example. Drops of an alcoholic solution of azobenzene containing 30·94 grams per litre were alternated with drops (1) of an alcoholic solution containing 0·16 molecule α-naphthol per litre, (2) of an alcoholic solution of the same substance containing 0·18 molecule per litre. In the first case the drops of the azobenzene solution increased in size, in the second case they decreased. A solution of azobenzene containing 30·94 grams per litre is therefore isotonic with a solution containing between 0·16 and 0·18 molecule of α-naphthol per litre. If the azobenzene solution were isotonic with the weaker of these α-naphthol solutions, and if these two substances are assumed to have the same isotonic coefficient, then the molecular weight of azobenzene would be $\frac{30{\cdot}94 \times 1}{{\cdot}16} = 193$. If, on the other hand, the azobenzene solution were isotonic with the stronger of the α-naphthol solutions, then the molecular weight of azobenzene would be $\frac{30{\cdot}94 \times 1}{{\cdot}18} = 172$. The conclusion, therefore, to be drawn from this experiment is, that the molecular weight of azobenzene lies between 172 and 193. The value corresponding to the formula for azobenzene is 182.

In some ways the method which has just been described recalls the hæmatocrit method of finding isotonic solutions. In both cases transference of the solvent takes place across a semi-permeable membrane; in Barger's arrangement, provided that the dissolved substances are non-volatile, the space between two neighbouring drops is permeable only to the molecules of the solvent, and is therefore equivalent to a semi-permeable membrane.

Osmotic Pressure and Boiling Point.—The boiling

point of a liquid is the temperature at which its vapour pressure is equal to the atmospheric pressure. Provided that we are dealing with a non-volatile solute, the vapour pressure curve for the solution lies below the vapour pressure curve for the pure solvent; hence, as shown graphically in Fig. 9, the solution must be raised to a higher temperature before its vapour pressure becomes equal to the atmospheric pressure; that is, the boiling point of the solution is higher than that of the solvent.

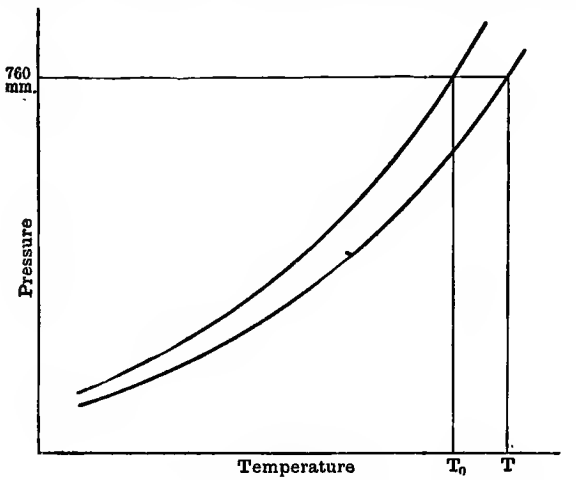

FIG. 9.

Further, the rise or elevation of the boiling point, $T-T_0$, is quantitatively related to the lowering of the vapour pressure, and therefore also to the osmotic pressure. The relation between the osmotic pressure of a moderately dilute solution and its boiling point is given by the formula $P=\frac{1000Sl}{24{\cdot}25}\cdot\frac{T-T_0}{T_0}$ atmospheres, where S is the specific gravity of the solvent at its boiling point, l is the latent heat of vaporisation for 1 gram of the solvent,

T_0 is its boiling point, and T that of the solution. In the case of an aqueous solution $S=0{\cdot}959$, $l=536$, and $T_0=373$, so that the osmotic pressure of an aqueous solution which boils $T-T_0$ degrees above the boiling point of water is $56{\cdot}8\ (T-T_0)$ atmospheres. If the solution boils, for instance, 0·1° higher than water, its osmotic pressure is 5·68 atmospheres.

Boiling Point and Molecular Weight.—In view of the existence of a quantitative relationship between the osmotic pressure and boiling point of a solution, it is obvious that there must be a definite connection also between the elevation of boiling point and the molecular weight of the dissolved substance. The nature of this connection may be deduced empirically in the following way.

Experiments have shown that the extent to which the boiling point of a given solvent is raised by the addition of a non-volatile substance is proportional to the concentration of that substance. This is borne out by the numbers in the following table,[1] those in the first column representing the weights of phenanthrene dissolved in each case in 22·75 grams of benzene, those in the second column giving the observed rise of the boiling point above that of pure benzene; the third column contains the ratios of the numbers in the first and second columns:—

Grams Phenanthrene.	Rise of Boiling Point.	Ratio.
0·619	0·389°	1·59
1·018	0·639°	1·59
1·648	1·023°	1·61

From these data, as well as from many others that might be quoted, it appears that the value of the ratio is practically constant, and we may therefore conclude

[1] Biltz, *Die Praxis der Molekelgewichtsbestimmung.*

that the rise of boiling point is proportional to the concentration of the solute.

Assuming that this rule is valid even for concentrated solutions, we may easily calculate what elevation would be observed if the solution under examination contained 1 gram-mol. of phenanthrene in some definite quantity (say 100 grams) of benzene. The first solution, for instance, quoted in the above table contains 0·619 gram of phenanthrene in 22·75 grams of benzene; this is the same as a solution containing $\frac{0{\cdot}619 \times 100}{22{\cdot}75} = 2{\cdot}72$ grams of phenanthrene per 100 grams of benzene. If this quantity of benzene contained a gram-mol.—178 grams—of phenanthrene, the corresponding elevation would be $\frac{0{\cdot}389 \times 178}{2{\cdot}72} = 25{\cdot}5°$. Suppose now another set of data, relating to a solution of phenyl benzoate in benzene, is similarly treated. A solution containing 1·015 gram of phenyl benzoate in 33·38 grams of benzene boils 0·387° higher than pure benzene. In this case the solution is the same as one containing $\frac{1{\cdot}015 \times 100}{33{\cdot}38} = 3{\cdot}04$ grams of phenyl benzoate per 100 grams of benzene. On the basis of proportionality between rise of boiling point and concentration, the elevation calculated for a solution containing 1 gram-mol.—198 grams—of phenyl benzoate in 100 grams of benzene would be $\frac{0{\cdot}387 \times 198}{3{\cdot}04} = 25{\cdot}2°$. This is very nearly the same figure as that calculated from the data for the phenanthrene solution, and instances of similar agreement might be multiplied.

It is found, in fact, that when experimental data for the boiling points of benzene solutions are used to calculate the elevation which would be produced by dissolving 1 gram-mol. of solute in 100 grams of benzene, the figures obtained in the majority of cases lie between

25° and 27°. This quantity appears therefore to be a characteristic constant for benzene, independent of the particular substance which is employed as solute; it is referred to as the 'molecular elevation of the boiling point' or as the 'boiling point constant.' In the case of benzene the figure which has been chosen as giving the best value for the molecular elevation of the boiling point is 26·7°. When the experimental data for the boiling points of solutions in water, alcohol, chloroform, &c., are treated in the same way as has been done for benzene, there is similarly obtained in each case a characteristic constant; the value of the molecular elevation of the boiling point (k) is 5·2° for water, 11·5° for ethyl alcohol, 21·0° for ether, and 39·0° for chloroform.

It is noteworthy that the values of k deduced by calculation from the experimental data can be confirmed. On theoretical grounds $k = \frac{0{\cdot}02T_0^2}{l}$, where T_0 is the boiling point of the solvent on the absolute scale, and l is its latent heat of vaporisation. The values of k calculated for various solvents by this formula are in good agreement with the figures quoted above.

When a trustworthy value has been obtained for k for any particular solvent, it is possible to determine the molecular weight of any new substance in this solvent. Suppose that a solution of g grams of this substance in 100 grams of the solvent is found to boil $t°$ higher than the pure solvent; if the molecular weight of the solute is M, then the molecular elevation of the boiling point will be $\frac{M}{g}t$, and this must be equal to k, which is already known. We have therefore $\frac{M}{g}t = k$, or $M = \frac{k \cdot g}{t}$. The following figures illustrate the appli-

cation of this formula. A solution containing 0·939 gram of a certain substance in 30 grams of benzene boils 0·588° higher than pure benzene. As benzene is the solvent, $k = 26·7°$; g, the weight of solute per 100 grams of the solvent, $= \frac{0·939 \times 00}{30} = 3·13$, so that $M = \frac{26·7 \times 3·13}{0·588} = 142$.

Practical Determination of the Rise of Boiling Point. Beckmann's Method.—A thermometer registers the same temperature when surrounded by the steam from boiling water as it does when surrounded by the steam from a boiling sugar or salt solution. In order therefore to find the rise of boiling point for such a solution, the bulb of the thermometer must be immersed (1) in boiling water, (2) in the solution boiling under the same conditions. A similar statement, naturally, applies to other solvents than water. It is found, however, that a thermometer immersed in a boiling liquid will register slightly different temperatures according to the way in which the heat is applied and the rate at which it is boiled. If the boiling vessel is in direct contact with a flame it is hardly possible to avoid superheating, and this leads to oscillation in the readings of the thermometer. Beckmann's apparatus, which is commonly used in determining the rise of boiling point, is designed to minimise these irregularities and to permit the comparison of solvent and solution under the same conditions.

One form of Beckmann's boiling point apparatus is represented in Fig. 10. The boiling tube A is provided with two side tubes t_1 and t_2, the former serving for the introduction of material into the boiling tube, the latter holding a condenser K. The lower end of A rests in a hole cut in the asbestos sheet L, which in its turn lies on the sheet of wire gauze D. The short glass cylinder G serves as an air jacket for A, and is covered with a

sheet of mica S. The upper end of the tube A is closed

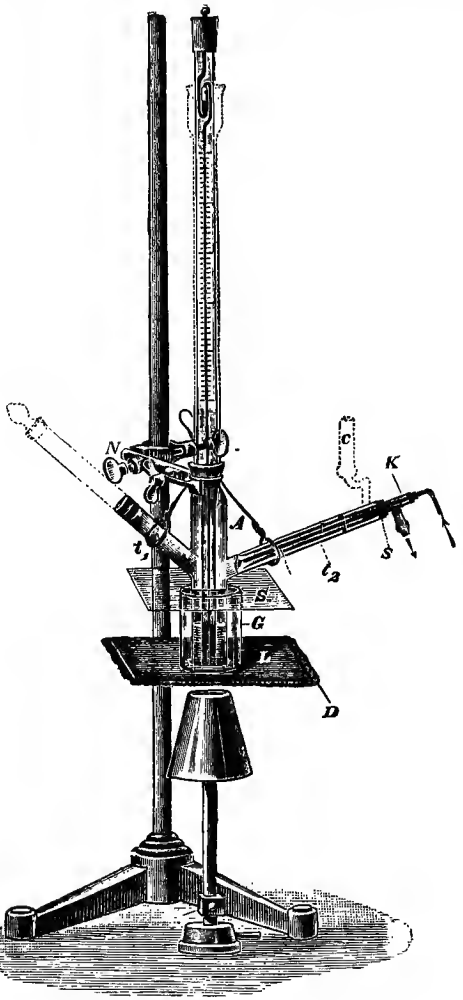

FIG. 10.

by a stopper, which carries the Beckmann thermometer.

When an experiment is to be made, the weight of the empty dry boiling tube is first ascertained. Enough solvent to cover the bulb of the thermometer is then introduced, and the tube is weighed again; the difference between the two weighings gives the weight of solvent taken. The apparatus is then set up, and the tube is heated by a small Bunsen flame. In order to ensure regular ebullition, and so avoid oscillations of temperature as far as possible, it is advisable to put some glass beads, garnets, or platinum foil in the boiling tube. According to Beckmann, platinum foil is most effective in promoting regular boiling, and he advises the use of 10–20 grams of platinum foil rolled up and cut so as to form small tetrahedra. The flame must be so adjusted that the liquid in the tube A boils freely; after it has boiled for 15–20 minutes the temperature ought to be practically constant, and readings of the thermometer made at minute intervals ought not to differ from a mean value by more than 0·01°. The constant temperature thus reached is taken as the boiling point of the solvent.

The burner is then put on one side, and after the apparatus has cooled a little, a weighed quantity of the solute is introduced into the boiling tube. If the solute is a solid substance, it is best to introduce it in the form of lumps, or pastilles made in a steel press; if the solute is liquid, a pipette shaped like a Sprengel pyknometer is employed. When the solute has been added, the burner is replaced under the boiling tube, the size of flame remaining unaltered. The solution is now boiled until a steady temperature is attained; the reading of the thermometer then recorded is taken as the boiling point of the solution. A fresh addition of solute may be made, and the corresponding boiling point determined in a similar manner. Since the boiling point of a liquid varies notably with the atmospheric pressure,

it is advisable to complete such a series of experiments in as short a time as possible.

The Beckmann Thermometer.—In a determination of the elevation of the boiling point it is not necessary to know the actual temperatures at which solvent and solution boil; it is sufficient to know accurately the *difference* in their boiling points. There is therefore no objection to varying the quantity of mercury in the working part of the thermometer, and thereby adapting it for use with solvents of widely different boiling points: the scale may then be made very open without the instrument becoming inconveniently large. In the Beckmann thermometer, which is commonly used for determining the rise of boiling point and depression of the freezing point, the tube is sealed at the bottom to a large bulb, and at the top to a reservoir in which any excess of mercury is kept. The scale of the instrument covers a range of about 6° Centigrade; the length corresponding to each degree is 3–5 cm., and each degree is divided into hundredths. The form of the reservoir will be understood by reference to the accompanying diagram of the thermometer head (Fig. 11).

FIG. 11.

When it is desired to alter the adjustment of the thermometer, the bulb is warmed so that the mercury expands a little way into the reservoir, as shown in the diagram. The mercury at the top of the reservoir may then be detached by tapping, so that the thermometer is now adjusted for a higher temperature than previously; or mercury may be jerked up from the bottom of the reservoir, so that the thermometer is adjusted for a lower temperature.

Landsberger's Apparatus for Determining Rise of Boiling Point.—When a liquid is boiled by direct contact with a flame, superheating to some extent is inevitable. In order to avoid this difficulty Landsberger suggested that a solution should be brought to its boiling point by passing in the vapour of the solvent. The vapour pressure of the solution, it must be remembered, is lower than that of the solvent at the same temperature, so that, for instance, when steam at 100° is passed into a salt solution at 100° the steam condenses, and by its latent heat of vaporisation raises the temperature of the salt solution above 100°, ultimately bringing it to its boiling point. In this case all risk of superheating is avoided, by virtue of the remarkable fact that the heating agent is at a *lower* temperature than the solution which is being boiled.

In Walker and Lumsden's modification of Landsberger's apparatus (see Fig. 12) the graduated boiling tube N is first charged with a small quantity of solvent, and this is boiled by passing in vapour through the tubes B and R from the flask F, where a large quantity of the solvent is boiled by direct heating. When the solvent in N is boiling the excess of vapour escapes through the small hole H, fills the space between the tubes N and E, and finally passes out into the condenser C. When the condensed solvent is dropping regularly from the end of the condenser the thermometer T is read, and the reading gives the boiling point of the solvent. The current of vapour is now stopped, and the most of the solvent which has condensed in N is poured back into F. A weighed quantity of the solute is introduced into N, and the current of vapour is restarted; when the solution is in active ebullition, and the condensed solvent is dropping from the end of the condenser at about the same rate as before, the ther-

mometer is read, and the current of vapour is stopped immediately. The thermometer T and the delivery tube R are removed, and the volume of solution in N ascertained as rapidly as possible. The boiling points of

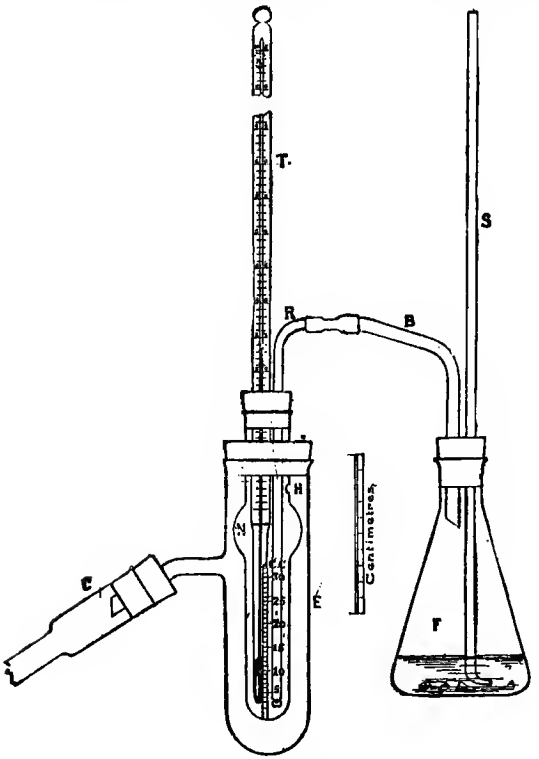

FIG. 12.

solvent and solution have thus been determined under the same conditions, and as the composition of the solution is known, all the data necessary for the calculation of the molecular weight are available.[1]

[1] For more details of this method, see *Journ. Chem. Soc.*, 1898, 73, 502; also Turner, *ibid.*, 1910, 97, 1184.

Osmotic Pressure and Freezing Point.—It is a well-known fact that the freezing point of a solution is in all ordinary instances lower than that of the pure solvent. That such must be the case can be shown by a consideration of the relative positions of the vapour pressure curves for solvent and solution; it is only necessary to take into account also the existence of a vapour pressure curve for the solid solvent. Below the freezing point the solid solvent has a tendency to pass into the state of vapour, and the measure of this tendency at each

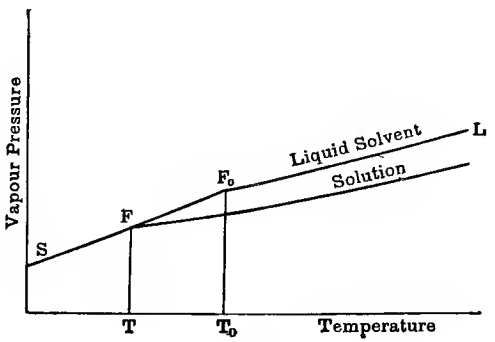

FIG. 13.

temperature is the vapour tension or vapour pressure. The vapour pressure curve for the solid is not a mere continuation of the vapour pressure curve for the liquid; the two are independent, as shown at F_0S and F_0L in Fig. 13. At the freezing point, however, the vapour pressures of solid and liquid must be equal, since that is the temperature at which the two are in equilibrium. The two curves must therefore intersect at the freezing point, and we may define the freezing point as the temperature at which the vapour pressure curve for the liquid intersects the vapour pressure curve for the solid. Similarly, the freezing point of a solution is the temperature at

which the vapour pressure curve for the solution cuts the vapour pressure curve for the solid solvent, and it appears from the relative position of the curves, as shown in Fig. 13, that F, the freezing point of the solution, must be at a lower temperature than F_0, the freezing point of the pure solvent.

This argument assumes that when a solution freezes it is pure solid solvent which crystallises out. This assumption is justified in the great majority of cases, and for aqueous salt solutions it can easily be shown that when freezing takes place pure ice separates out. A test-tube containing $\frac{N}{1000}KMnO_4$ is kept in a freezing mixture for a short time until the layer next the glass has frozen; the tube is then set in a wider jacket tube and again immersed in the freezing mixture; in this way the freezing of the permanganate solution proceeds more slowly. When the contents of the tube have solidified completely it is seen that the coloured salt has been concentrated in the centre of the test-tube, and is surrounded by an envelope of perfectly colourless ice.

The extent to which the freezing point of a solution is lower than that of the solvent, the depression of the freezing point, as it is called, depends then on the vapour pressure of the solution, and must therefore be quantitatively related to the osmotic pressure. The relation between osmotic pressure P and freezing point is given by the formula $P=\frac{1000S\omega}{24{\cdot}25}\cdot\frac{T_0-T}{T_0}$ atmos., where S is the specific gravity, T_0 the freezing point, and ω the latent heat of fusion of the solvent, while T is the freezing point of the solution. If the solvent is water, then $S=1$, $\omega=79{\cdot}6$, and $T_0=273$, and $\frac{1000S\omega}{24{\cdot}25T_0}=12{\cdot}03$, so that the osmotic pressure of an aqueous solution is

given by the formula $P=12{\cdot}03(T_0 - T)$ atmos. The mean value, for instance, which has been found by various investigators for the freezing point of a 1 per cent. sucrose solution is $-0{\cdot}0546°$. The osmotic pressure of this sucrose solution at 0° C., calculated by the foregoing formula, would therefore be 0·656 atmosphere, in good agreement with the value (0·649) found by Pfeffer.

Freezing Point and Molecular Weight.—The relationship existing between the freezing point of a solution and its osmotic pressure involves another between the freezing point and the molecular weight of the dissolved substance. What there is to be said in this connection is very similar to what has already been said about the boiling point; elevation of the boiling point and depression of the freezing point are comparable quantities.

The depression of the freezing point for a solution is proportional to the concentration of the dissolved substance. This statement embodies the results of countless observations, and may be illustrated by the following data for the depression of the freezing point in aqueous solutions of chloral hydrate:—

Grams Chloral Hydrate in 100 grams Water.	Depression of Freezing Point.	Ratio.
2·834	0·335°	8·4
4·878	0·575°	8·5
6·595	0·775°	8·5

If on the basis of proportionality between freezing point depression and concentration we calculate what would be the depression for a solution containing 1 gram-mol. (165·5 grams) of chloral hydrate per 100 grams of water, we obtain for the three solutions quoted the values 19·6°, 19·5°, 19·4° respectively. If the experimental data for solutions of other non-electrolytes in water are similarly treated, the value found for the depression which would be produced by 1 gram-mol.

of solute in 100 grams of water is not very different from the figures just quoted. A solution, for instance, containing 0·609 gram of ethyl alcohol in 100 grams of water freezes at −0·243°; the depression for a gram-mol. would be $\frac{0{\cdot}243 \times 46}{0{\cdot}609} = 18{\cdot}4°$. It appears therefore that the figure for the depression due to 1 gram-mol. of solute in 100 grams of solvent is a characteristic constant for each solvent, and it is described as the 'molecular depression of the freezing point,' or, shortly, as the 'freezing point constant.' The accepted value of this constant (k) for water is 18·6°, for acetic acid 39·0°, and for benzene 50·0°. On theoretical grounds $k = \frac{\cdot 02 T_0^2}{\omega}$, where T_0 is the freezing point of the solvent on the absolute scale, and ω is the latent heat of fusion. It is interesting to note that the values thus calculated for k are in good agreement with those deduced from the consideration of the observed depressions.

The value of k which has been adopted for any solvent after a study of various solutes of known molecular weight may be employed in determining the molecular weight of a new substance. Suppose that for a solution of this new substance containing g grams per 100 grams of solvent the observed depression of freezing point is $t°$; if the required molecular weight of the solute is M, then the molecular depression of the freezing point would be $\frac{M}{g}t$, and this must be equal to k, which is already known; that is, $\frac{M}{g}t = k$, or $M = \frac{k \cdot g}{t}$. An illustration of the application of this formula may be quoted. A solution containing 0·565 of a certain substance in 23·4 grams of water freezes at −0·77°; it is required to calculate the molecular weight of this substance. The value of g in this

case is $\frac{0{\cdot}565 \times 100}{23{\cdot}4} = 2{\cdot}415$, and since k for water $= 18{\cdot}6°$, $M = \frac{18{\cdot}6 \times 2{\cdot}415}{0{\cdot}77} = 58{\cdot}3$.

Experimental Determination of the Depression of the Freezing Point.—The apparatus chiefly employed for this purpose was devised by Beckmann, and is represented in Fig. 14. It consists of a tube A, set in a jacket tube B, and provided with a Beckmann thermometer D, and a platinum or nickel wire stirrer. The jacket tube B rests in a metal plate, which forms the cover of the thick glass jar C. When an experiment is to be made the glass jar is filled with a suitable cooling mixture; the mixture should be such that its temperature is not more than 2°–3° below the freezing point of the solvent to be used. A known weight of solvent is put in the tube A, and the cork carrying thermometer and stirrer is inserted; the thermometer has been previously adjusted, so that at the freezing point of the solvent the top of the mercury thread is somewhere on the upper part of the scale. The tube A is first immersed directly in the cooling mixture, and only when the temperature has fallen nearly to the freezing point of the solvent is it set inside the jacket tube. The contents

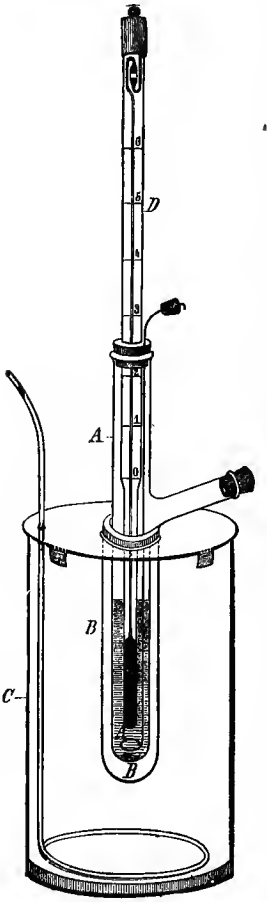

FIG. 14.

of A are stirred regularly, and the temperature falls steadily. Close observation of the thermometer shows that after a short time the mercury ceases to fall, then rises and remains steady at one point; the temperature thus marked is the freezing point of the solvent. The tube A is now taken out of the bath and a weighed quantity of solute is introduced through the side tube; after it has completely dissolved, the operation of taking the freezing point is carried out as before. The amount of supercooling, that is, the interval of temperature between the lowest point to which the mercury falls and the highest point to which it rises (the freezing point, in other words), should be noted; if it is greater than 0·4°–0·5°, the determination of the freezing point should be repeated, and the occurrence of excessive supercooling avoided by introducing, if necessary, a tiny crystal of the solvent. Excessive supercooling involves the separation of a considerable quantity of the solid solvent when freezing occurs, and this would mean an appreciable increase in the concentration of the solution. When the freezing point of the first solution has been satisfactorily determined, a further quantity of solute may be introduced, and the new freezing point ascertained as before. For each addition of solute there is a corresponding depression, and from each pair of values the molecular weight of the dissolved substance may be calculated by the formula $M=\frac{k \cdot g}{t}$, already discussed. The value obtained for M by this method ought not to differ from the correct value by more than 3–5 per cent.

In the most accurate work it is necessary to adjust the temperature of the external bath so that it is only slightly below the freezing point of the solution in A. This is conveniently done for aqueous solutions by putting

ether in the external bath and aspirating a current of air through it.[1] By regulating the current of air, any desired temperature between 0° and −15° is easily maintained.

Biological Applications.—The difficulties involved in the direct determination of osmotic pressure have been repeatedly emphasised. In the depression of the freezing point, however, we have a measure of the osmotic pressure of a solution which is more accessible by ordinary experimental work, and the freezing point method has therefore been extensively applied in studying the osmotic power of various fluids occurring in the living organism. It should however be pointed out that the indirect determination of osmotic pressure by means of the freezing point is, in one sense, much less accurate than the process of direct measurement. For, as has already been shown, the osmotic pressure P of an aqueous solution is related to the freezing point depression $T_0 - T$ by the formula $P = 12{\cdot}03(T_0 - T)$ atmos., and it appears from this that to a freezing point depression of 0·001°, which it is very difficult to measure with any approach to accuracy, there corresponds an osmotic pressure of 9 mm. of mercury, a quantity which can be accurately determined. In the absence, however, of trustworthy and rapidly acting semi-permeable membranes the more practical if less accurate freezing point method of studying osmotic pressure is used by the ordinary worker.

In the ordinary form of the Beckmann freezing point apparatus 10–20 cub. cm. of liquid are required for a determination. It is sometimes difficult, however, if not impossible, to obtain this quantity of a fluid from an organism, and hence if the osmotic value for such a fluid is to be determined by the freezing point method,

[1] Raoult, *Zeit. physikal. Chem.*, 1898, 27, 617.

an apparatus permitting the use of a smaller quantity of liquid is desirable. With this end in view, modified forms of apparatus have been introduced, such as that described by Guye and Bogdan,[1] which differs from the Beckmann apparatus chiefly in having a smaller freezing tube and a smaller thermometer bulb. An experiment can be carried out with 1·5 cub. cm. of liquid, and the authors claim that the results are nearly as accurate as those obtained with the usual apparatus under ordinary working conditions.

The freezing point method has been extensively used in studying the osmotic pressure of the blood from different animals, and the variations in this pressure resulting from changes in the external conditions. It is immaterial whether defibrinated blood or blood serum is taken for the determination of the freezing point, since the corpuscles, like other suspended particles, have no influence on the osmostic pressure. Further, blood plasma and blood serum have practically the same freezing point, a little proteid more or less making no appreciable difference.

Numerous investigations have shown that the freezing point of mammalian blood does not vary much from one species to another. This is brought out by the following figures:—

Animal.	Freezing Point of Blood.
Man	−0·56
Ox	−0·58
Horse	−0·56
Rabbit	−0·59
Cat	−0·63
Dog	−0·57
Sheep	−0·62

Nor is there much variation from time to time in the

[1] *Journ. chim. phys.*, 1903, **1**, 379.

osmotic pressure of the blood of one individual, a fact that may be contrasted with the behaviour of urine in this respect. Even in the case of a healthy person, the freezing point of the urine varies within very wide limits in the course of twenty-four hours, and, according to Bouchard,[1] while the normal freezing point of undiluted urine may be taken as about $-1{\cdot}35°$, it may vary from $-0{\cdot}50°$ to $-2{\cdot}24°$ in different pathological conditions.

Another direction in which the freezing point method has been applied is in the study of the relation between the osmotic pressure of the blood of fishes and that of the surrounding medium. In the case of all invertebrate marine animals the freezing point of the blood or body fluid is the same as that of the water in which they live. Further, these organisms are unable to preserve any difference between the osmotic pressure of their body fluid and that of the surrounding medium; when the osmotic pressure of the latter is artificially varied by dilution or concentration the body fluid undergoes a corresponding change, as shown by the freezing point. This is illustrated by the figures in the following table, bearing on the behaviour of a species of crab (*Maia verrucosa*). The figures under I. are the freezing points of sea-water (normal and artificially modified), while the figures under II. are the freezing points of the body fluid of the crab after immersion for some time in the corresponding water:—

	I.	I.
Normal sea-water	$-2{\cdot}3°$	$-2{\cdot}3°$
Concentrated sea-water . . .	$-2{\cdot}98°$	$-2{\cdot}9°$
Diluted sea-water	$-1{\cdot}38°$	$-1{\cdot}4°$

It is obvious that the organism is unable to regulate the osmotic pressure of its body fluid.

In the case, however, of many marine vertebrates

[1] *Compt. rend*, 1899, 128, 64.

the osmotic pressure of the blood or body fluid is quite different from that of the surrounding medium, and variation in the osmotic pressure of the latter is accompanied by only a slight variation of the former. This point is well illustrated by some observations of Dakin[1] on the blood of fish taken from sea-water of naturally varying concentration. He determined the freezing point of the blood of plaice caught in Kiel harbour, in the open Baltic, in the Kattegat, and off Helgoland. The freezing points of the water in the four cases were respectively $-1{\cdot}09°$, $-1{\cdot}30°$, $-1{\cdot}66°$, and $-1{\cdot}90°$, while the freezing points of the blood of the plaice were $-0{\cdot}66°$, $-0{\cdot}72°$, $-0{\cdot}73°$, $-0{\cdot}79°$. The osmotic pressure, therefore, of the blood of the plaice is dependent only to a very limited extent on the osmotic pressure of the surrounding medium. The codfish is still more independent, and any variation observed in the freezing point of the blood in this case is covered by individual differences. With elasmobranchs, on the other hand, the osmotic pressure of the blood is almost the same as that of the surrounding sea-water, and as this increases in density, so the osmotic pressure of the blood changes. It is found in general that the freezing point of the blood of marine teleosts taken from the North Sea is on the average about $-0{\cdot}75°$, whilst that of the blood of fresh-water teleosts averages about $-0{\cdot}53°$; in each case the organism is largely independent, so far as osmotic pressure is concerned, of the medium in which it lives.

[1] *Biochem. Journ.*, 1908, 3, 258, 473.

CHAPTER VII

THE BEHAVIOUR OF SALTS, ACIDS, AND BASES IN AQUEOUS SOLUTION

Facts apparently Inconsistent with Avogadro's Hypothesis.—The acceptance of Avogadro's hypothesis was retarded by the discovery of certain cases in which the molecular weight of a substance, deduced from its vapour density, was quite out of harmony with the formula which seemed probable on grounds of chemical analogy. The vapour density of ammonium chloride, for instance, is only about half what it ought to be if NH_4Cl is the correct formula for this compound; on the other hand, the vapour density of acetic acid has a value greater than corresponds to the formula $CH_3.COOH$. In the extension of Avogadro's hypothesis to solutions similar difficulties have been encountered. Cases are known in which the molecular weight of a dissolved substance, calculated from the depression of the freezing point or one of the other osmotic properties, is greater than the value which corresponds with the ordinarily accepted formula. Just as the vapour density of acetic acid is abnormally high, so the molecular weight of acetic acid dissolved in benzene, deduced from its influence on the freezing point of benzene, is nearly double the value which corresponds to the formula $CH_3.COOH$. High values are similarly obtained for the molecular weight in benzene solution of all substances containing the $-OH$ group, phenol and alcohol, for instance. A glance at

the formula by which molecular weight is calculated from the depression of the freezing point, $M = \frac{k \cdot g}{t}$ (see p. 107), shows that an abnormally large value for the molecular weight is the result of an abnormally small depression. Now the depression of the freezing point, like the osmotic pressure, is a measure of the number of dissolved units, and hence an abnormally small depression points to a reduction in the number of dissolved units below the figure which we should expect from the amount of substance actually in solution. Such a reduction must be due to the clubbing together, or association, of the normal molecules to form larger aggregates.

Abnormally Great Depressions of the Freezing Point.—More interesting perhaps are the cases, the deviation of which from the normal is in the opposite direction. There are very many substances the molecular weights of which, calculated from their influence on the freezing point of water, are quite inconsistent with the accepted formulæ. A solution of sodium chloride, for instance, containing 1·135 gram of the salt in 100 grams of water, freezes at $-0{\cdot}687°$. Taking $k = 18{\cdot}6°$ for water (see p. 107), and calculating the molecular weight of the dissolved substance in the usual way, we obtain $M = \frac{18{\cdot}6 \times 1{\cdot}135}{0{\cdot}687} = 30{\cdot}7$, a value far below 58·5, which is the molecular weight for sodium chloride, on the assumption that it contains one atom of sodium and one of chlorine. In the case of other salts also, as well as for many acids and bases, there is an equally marked discrepancy between the accepted formula of the substance and the molecular weight deduced from its osmotic behaviour. A more definite conception of the extent of the discrepancy will be gained by a glance at the figures in the following tables. In the first column of

each table is recorded the strength of the solution in gram-molecules per litre; the second column contains the observed depressions t, and the third column contains the values $i=\frac{t}{t_0}$, t_0 being the depression which would be observed if the solute behaved normally:—

Sodium Chloride.			Sodium Sulphate.		
Concentration.	t.	$i=\frac{t}{t_0}$.	Concentration.	t.	$i=\frac{t}{t_0}$.
0·117	0·424°	1·93	0·028	0·141°	2·66
0·194	0·687°	1·87	0·070	0·326°	2·46
0·324	1·135°	1·86	0·117	0·515°	2·33
0·539	1·894°	1·85	0·195	0·817°	2·21

These figures, and many others which might be quoted, show that the depression of the freezing point of water caused by salts is abnormally great, a fact that points to an increase in the number of dissolved units above the figure which we should expect from the amount of salt actually present in any solution. From the figures obtained by Arrhenius,[1] it appears that for salts of the type of sodium chloride the values of i run up to 2, while for salts, such as sodium and potassium sulphates, magnesium, calcium and strontium chlorides, the values run up to 3. Evidence of this enhanced osmotic activity on the part of salts is found also in the values of the isotonic coefficients tabulated on p. 72. The isotonic coefficients, it must be remembered, represent the relative osmotic pressures of equimolecular solutions, and the figures for the isotonic coefficients show that sodium chloride and potassium nitrate exhibit an osmotic activity which is about 1·7 times as great, while calcium chloride exhibits an osmotic activity which is 2·3—2·4 times as great as that of sucrose taken as the normal substance.

As already indicated, this enhanced osmotic activity

[1] *Zeit. physikal. Chem.*, 1888, **2**, 491.

on the part of salts points to an abnormally large number of dissolved units in their solutions. How is this to be explained? Reference has been made to the fact that ammonium chloride and other substances are found to have vapour densities much below the values corresponding to their accepted formulæ. This exceptional behaviour has been reconciled with Avogadro's hypothesis by assuming a dissociation of the vaporised molecule into two or more simpler molecules; in the case of ammonium chloride, indeed, direct evidence is obtainable, showing that when the substance is vaporised it breaks up into ammonia and hydrogen chloride, giving two molecules in place of one. Between the case of these abnormally low vapour densities and the case of abnormally great depressions of the freezing point of water there is a historical parallel in that a dissociation hypothesis has been brought forward also to account for the exceptional osmotic behaviour of salts (acids and bases) in aqueous solution.

The Electrolytic Dissociation Hypothesis.—In 1887 Arrhenius propounded the view[1] that acids, bases, and salts in aqueous solution are dissociated to a greater or less extent into positively and negatively charged particles or 'ions,' and that the increase in the number of units in solution which arises from this dissociation is responsible for the abnormally high osmotic activity of these substances. Sodium chloride, according to this hypothesis, splits up to a large extent, when dissolved in water, into positively charged sodium ions $\overset{+}{\mathrm{Na}}$, and negatively charged chlorine ions $\overline{\mathrm{Cl}}$; potassium nitrate similarly dissociates into $\overset{+}{\mathrm{K}}$ and $\overline{\mathrm{NO_3}}$.[2] In both these

[1] *Zeit. physikal. Chem.*, 1887, **1**, 631.

[2] Positive ions are very frequently indicated by dots, negative ions by dashes; thus—$Na^{\cdot}$, $H^{\cdot}$, $NH_4^{\cdot}$, Cl', NO_3'.

cases, as also in others, such as hydrochloric acid ($\overset{+}{H}$, $\overline{Cl}$), potassium hydroxide ($\overset{+}{K}$, $\overline{OH}$), potassium acetate ($\overset{+}{K}$, $\overline{CH_3.COO}$), and ammonium chloride ($\overset{+}{NH_4}$, $\overline{Cl}$), one molecule produces two ions, so that even on the supposition of complete dissociation the abnormal osmotic effect of these compounds, whether measured by the lowering of vapour pressure, the elevation of the boiling point, or the depression of the freezing point of water, cannot be greater than twice the effect produced by an equimolecular quantity of a normal substance. In harmony with this it is found, as already stated, that the values of i for sodium chloride, potassium nitrate, and the like, run up to 2. According to Arrhenius, sodium sulphate in aqueous solution is more or less dissociated into three ions, $\overset{+}{Na}$, $\overset{+}{Na}$, and $\overline{SO_4}$, the last carrying a double negative charge; similarly, calcium chloride produces three ions, one with a double positive charge $\overset{++}{Ca}$, and two with a single negative charge $\overline{Cl}$, $\overline{Cl}$. In both these cases, and in analogous compounds, one molecule produces three ions, and on the supposition of complete dissociation the abnormal osmotic effect would be three times the effect due to an equimolecular quantity of a normal substance. In harmony with this it is found that the values of i for sodium and potassium sulphates, magnesium, calcium, and strontium chlorides, and other analogous compounds, run up to 3.

If this hypothesis of the ionic dissociation of salts, acids, and bases in aqueous solution is accepted, then we can deduce the degree or extent of the dissociation in any solution by comparing the observed depression t of the freezing point with the depression t_0 which would be observed were there no dissociation; the latter value is calculated by the formula $t_0 = \frac{k.g}{M}$, in which M is the

normal molecular weight of the dissolved substance. Suppose that of 100 molecules of a dissolved salt the fraction a has undergone dissociation, each dissociated molecule producing n ions, then there remain in the undissociated condition $100(1-a)$ molecules. The number of molecules which have undergone dissociation is $100a$, and the number of ions so produced is $100a \times n$; the total number of units in solution is therefore $100(1-a)+100an$, and the actually observed depression t of the freezing point must be proportional to this figure. If, on the other hand, there were no dissociation the number of units in solution would be merely 100, and to this figure there would correspond the depression t_0. Since the depressions are proportional to the numbers of units present in solution, $\frac{t}{t_0}=\frac{100(1-a)+100an}{100}=1+(n-1)a$, so that $a=\frac{t-t_0}{(n-1)t_0}$; or if $\frac{t}{t_0}$ be expressed by the symbol i, $a=\frac{i-1}{n-1}$. If this formula is applied to the data bearing on the freezing points of sodium chloride and sodium sulphate solutions (see p. 116), it appears that in a sodium chloride solution containing 0·2 of a gram-mol. per litre, 85–90 per cent. of the salt is dissociated into its ions, and in a sodium sulphate solution of the same strength about 60 per cent. of the salt is so dissociated. It appears also from the figures on p. 116 that the more dilute the solution the greater is the percentage of salt in the dissociated condition.

Ionic Dissociation and Electrolytic Conduction.—The claim which the hypothesis of ionic dissociation makes on our consideration is greatly strengthened by the fact that it not only furnishes an explanation of the abnormal osmotic influence of acids, bases, and salts in aqueous solution, but gives also an intelligible interpretation of

various other phenomena. It is well known that a solution of sugar or alcohol in water is no better a conductor of the electric current than water itself; sugar and alcohol are non-electrolytes. On the other hand, there are many substances the aqueous solutions of which are relatively good conductors of the electric current. As Arrhenius pointed out, these are precisely the substances which have an abnormally great effect in raising the boiling point or lowering the freezing point of water. Sugar and alcohol are non-electrolytes; their effect on the freezing point of water is normal. Sodium chloride, potassium nitrate, hydrochloric and sulphuric acids are electrolytes; their aqueous solutions conduct the electric current, and they undergo decomposition under the influence of the current; they are also among the substances which produce an abnormal depression of the freezing point.

All this becomes intelligible if it is supposed that this latter class of substances is liable to ionic dissociation. For, according to Arrhenius's hypothesis, a solution of sodium chloride, to take one of the substances which have an abnormal influence on the freezing point of water, contains a large proportion of dissociated molecules in the form of positively and negatively charged ions. Accordingly when two electrodes, one charged positively and the other negatively, are immersed in such a solution, an attractive force is exerted on the ions of opposite sign. Under the influence of this force the positively charged ions move towards the negative electrode, and the negatively charged ions towards the positive electrode. The passage of an electric current, then, through a solution of sodium chloride or any other electrolyte consists in a streaming of positive ions in one direction and of negative ions in the opposite direction. The neutral or undis-

sociated molecules are unaffected; they are not charged, and experience no impulse to move rather in one direction than another; they are inactive so far as the transport of electricity through the solution is concerned.

On the basis of this view, the efficiency of a given quantity of a salt in conducting the current must depend on the extent to which the salt is dissociated; if the degree of dissociation is high, then the proportion of current-carriers will be high also, and the power of conducting the current, the conductivity as it is called, will be relatively great. A solution of a substance, on the other hand, which is ionised only to a small extent, will be a relatively poor conductor of the electric current. It is further obvious that if we could compare the conductivity of an actual sodium chloride solution with the conductivity which the same amount of the salt would exhibit if it were completely ionised, we should obtain a measure of the dissociation in the actual solution.

Increase of Conductivity with Dilution.—The figures recorded on p. 116 for solutions of sodium chloride and sodium sulphate show that the degree of dissociation increases as the solutions become less concentrated, *i.e.* as the dilution increases, and this is a statement that applies to dilute aqueous solutions of all electrolytes, so far as the freezing point evidence goes. It is therefore to be expected, on the basis of the ionic dissociation hypothesis, that for a given quantity of a salt the conducting efficiency—the conductivity—should become greater as the concentration of the solution decreases. This is what actually takes place, as can be demonstrated by the following simple experiment:—

A rectangular glass jar is procured, say about 25 cm. high, 4 cm. wide, and 10 cm. long, and two strips of sheet

copper are cut to fit the opposite ends of the jar from top to bottom; the top ends of the strips should project somewhat beyond the mouth of the jar, and are provided with binding screws. The two strips are kept pressed up against the opposite ends of the jar by glass rods, the ends of which are inserted into rubber stoppers, the total length of the rods + the stoppers being adjusted to the distance between the strips. A little concentrated sodium acetate solution is introduced into the jar, and the latter put in series with a sulphuric acid voltameter, fitted with a delivery tube so that the gas liberated when a current is passing may be collected in an inverted tube filled with water. In this arrangement the rate at which the bubbles of gas pass up the tube is roughly a measure of the strength of the current passing through the voltameter and any other piece of apparatus which is in series with it. A current is now sent through the jar and the voltameter, and is so regulated that a bubble of gas ascends in the water tube once in two seconds or thereabouts. As soon as the current is adjusted, distilled water is poured continuously into the jar until it is full. In this way the sodium acetate solution is diluted without altering the quantity of salt which is between the electrodes, and which is therefore available for conduction of the current. This progressive dilution of the salt solution is accompanied by a gradual increase in the rate of evolution of gas from the voltameter, which points to an increase in the strength of the current which is passing. The resistance, therefore, which the current experiences between the electrodes in the jar is diminished by dilution of the sodium acetate solution; that is, the conducting efficiency of the salt which is between the electrodes, and which is constant in quantity throughout the experiment, increases with dilution. If a concentrated acetic acid solution is put in the jar instead of sodium

acetate solution, and the current is suitably adjusted, a similar result is obtained, except that the increase in the rate of evolution of the bubbles with dilution is much more marked in this case. The increase of conductivity with dilution is therefore more rapid for acetic acid than for sodium acetate.

Measurement of Conductivity.—The experiment just described demonstrates qualitatively the increase of conductivity with dilution, but it is easy to get a quantitative

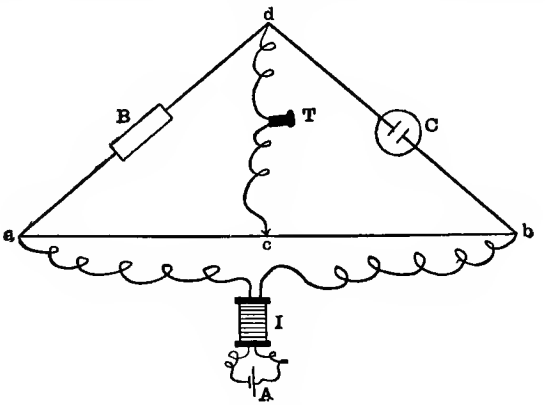

FIG. 15.

measure of this change by determining the conductivity. The determination of the conductivity of a solution resolves itself into the determination of the resistance, and this is effected by a modification of the ordinary Wheatstone bridge method. The arrangement of the apparatus necessary for the determination of the resistance of a solution is represented diagrammatically in Fig. 15, where B is a resistance box, C is a cell containing the solution; *ab* is the bridge wire. The ends *a* and *b* of the bridge wire are connected with the small induction coil I, which gives an alternating current. This is neces-

sary in order to avoid the polarisation effects which would make themselves felt if a continuous current, such as is usually employed in the Wheatstone bridge method, were passed through the solution in C. The use of an alternating current necessitates the replacement of the ordinary galvanometer by an instrument which will respond to such a current. A telephone is usually employed, and, as shown at T in the diagram, it is connected on the one hand with the point *d*, and on the other with the moving contact *c*. When the apparatus is ready, the induction coil is operated by the accumulator A, and the moving contact is so adjusted on the wire *ab* that there is no sound in the telephone. It follows then, according to well-known principles, that

$$\frac{\text{Resistance in C}}{\text{Resistance in B}} = \frac{\text{Length } bc}{\text{Length } ac};$$

as the resistance in B is known and the lengths *ac* and *bc* are easily ascertained on the scale over which the bridge wire is stretched, the resistance of the cell can be calculated.

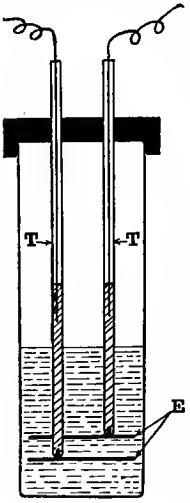

FIG. 16.

Various types of cell are employed according as the resistance of the solution is high or low. One of the most generally serviceable is shown in Fig. 16. The electrodes E are made of stout platinum foil, and are in electrical connection with the mercury in the glass tubes TT by means of two pieces of stout platinum wire sealed through the ends of these tubes. Connection is made with the rest of the apparatus by copper wires which dip in the mercury. The tubes TT are fitted tightly in the ebonite lid of the cell, so that the electrodes may be lifted out, rinsed and dried without their relative

position being altered in the slightest. Before the cell is used the electrodes are platinised, that is, coated with a fine deposit of platinum black. This is done by electrolysing a solution of platinic chloride in the cell, the electrodes being made alternately cathode and anode.

It is customary to compare the resistance and conductivity of solutions with the resistance and conductivity of a hypothetical liquid which, if enclosed in a centimetre cube, would offer a resistance of 1 ohm between two opposite faces of the cube acting as electrodes. In dealing with the density of liquids and solids we take water as the standard, and speak then of the *specific* gravity of a substance; similarly, in dealing with the resistance and conductivity of solutions we take the said hypothetical liquid as the standard, and speak then of the specific resistance and the specific conductivity of a solution. The specific resistance of a solution therefore is simply the number which represents the resistance in ohms of a column of the solution 1 sq. cm. in section and 1 cm. long. The actual conducting column in the cell C has the resistance r, and if we suppose that the distance between the electrodes is l cm., and that the section of the conducting column is s sq. cm., then R, the specific resistance of the solution, is obtained by the formula $R=r\frac{s}{l}$. The conductivity of a solution is the reciprocal of the resistance, so that if we take κ to represent specific conductivity, we have $\kappa=\frac{1}{R}=\frac{1}{r}\cdot\frac{l}{s}$. The evaluation of the specific conductivity depends therefore on the dimensions of the cell used, as well as on the observed resistance r. For most ordinary cells it would be impossible to obtain the exact values of l and s by mere measurements of length, and the device is usually adopted of first charging the cell with a solution the

specific conductivity κ_0 of which has been accurately determined by special experiments. Such a solution is $\frac{N}{50}$KCl, for which $\kappa_0 = 0{\cdot}002768$ at 25°, or $\frac{N}{100}$KCl, for which $\kappa_0 = 0{\cdot}001412$ at 25°. These exact values have been obtained by determining the resistance of the solutions in cells for which l and s could be accurately determined. Suppose, then, that in an actual experiment the cell is charged first with $\frac{N}{50}$KCl, and secondly with the solution, the specific conductivity (κ) of which is to be found; further, that the resistances measured in the two cases are respectively r_0 and r at 25°. Subject to the condition that the relative position of the electrodes has remained the same throughout the experiment, $\kappa_0 = \frac{1}{r_0} \cdot \frac{l}{s}$ and $\kappa = \frac{1}{r} \cdot \frac{l}{s}$, whence it follows that $\kappa = \kappa_0 \cdot \frac{r_0}{r}$. The value of κ_0 is known, the values of r_0 and r have been determined, so that κ is obtained by a simple calculation.

It ought to be pointed out here that all solutions used in determinations of conductivity are prepared with specially pure water. Ordinary distilled water is redistilled under certain conditions, and thus freed from various impurities which add to its conducting power. The question as to how far this purification of water can be carried will be discussed later (p. 170).

Specific Conductivity and Equivalent Conductivity.—When solutions of a salt of gradually decreasing concentration are examined, it is found that the specific conductivity regularly diminishes. This falling off in the specific conductivity with increasing dilution is illustrated by the figures in the first two columns of the following table, which refers to sodium chloride. The

figures in the first column are the concentrations of the salt in gram-equivalents per litre of solution, those in the second column are the corresponding values of the specific conductivity at 25°; the significance of the figures in the third column will be explained presently.

Concentration.	κ.	λ.
0·0312	0·00356	114·1
0·0156	0·00183	117·4
0·0078	0·000938	120·1
0·0039	0·000476	122·0

That the specific conductivity should diminish with increasing dilution is only to be expected, for its value is in each case based on the resistance between two opposite faces of a centimetre cube filled with the solution. Now as the solution is gradually diluted there will be less and less of the salt in this centimetre cube, less and less therefore of the substance which acts as the carrier of the current, for the water is a non-conductor, or at all events an exceedingly poor conductor. It is quite natural, then, that specific conductivity, based as it is on the consideration of a definite volume of the solution, should diminish with dilution.

If, however, we are to ascertain really how the efficiency of a given salt in conducting the current varies with dilution, we must obtain a set of figures which relate to the same quantity of the salt at each dilution. Such figures are directly deducible from the values for the specific conductivity. Suppose that we are dealing with a normal solution of sodium chloride, and that we have an electrolytic cell the sides of which are formed by electrodes 1000 sq. cm. in area and exactly 1 cm. apart. This cell would hold 1 litre of the normal solution, and the quantity of salt between the electrodes would be 1 gram-equivalent. Since this solution may be regarded as made up of 1000 centimetre cubes, the

figure which represents the conducting power of the 1 gram-equivalent of salt will be 1000 times the figure which stands for the conductivity of a centimetre cube of the solution, that is, 1000κ, where κ is the specific conductivity of a normal sodium chloride solution. If, instead of a normal solution of sodium chloride, we are dealing with a half-normal solution, the volume of the latter containing 1 gram-equivalent of salt is 2000 cub. cm., and to bring this gram-equivalent between two electrodes which are 1 cm. apart, the area of each electrode would have to be 2000 sq. cm. The bulk of solution between the electrodes in such an imaginary cell might be regarded as made up of 2000 centimetre cubes, and the figure which represents the conducting power of the 1 gram-equivalent of salt will be 2000 times the figure which stands for the conductivity of a centimetre cube of the solution, that is, 2000κ, where κ is the specific conductivity of a half-normal sodium chloride solution. This argument might obviously be extended to cover any solution of sodium chloride or any other electrolyte, but enough has been said to show that a measure of the conducting power of 1 gram-equivalent of electrolyte at various dilutions is to be found in the product—specific conductivity × volume of solution in cub. cm. which contains 1 gram-equivalent. For this volume of solution the symbol ϕ is generally taken, and the conducting power of a gram-equivalent, the *equivalent conductivity* as it is termed, is represented by the symbol λ, so that $\lambda = \kappa \cdot \phi$.

With increasing dilution, as already indicated, the specific conductivity diminishes; the equivalent conductivity, on the other hand, increases steadily. This statement is borne out by the figures in the third column of the table on p. 127, and in greater detail by the numbers contained in the following table:—

EQUIVALENT CONDUCTIVITY AT 18°.

Gram-equivalents per litre.	KCl.	$CH_3.COONa$.	HCl.	NaOH.	$CH_3.COOH$.
1·0	98·3	41·2	301	160	1·32
0·5	102·4	49·4	327	172	2·01
0·1	112·0	61·1	351	183	4·60
0·05	115·9	64·2	360	190	6·48
0·01	122·4	70·2	370	200	14·3
0·005	124·4	72·4	373	203	20·0
0·002	126·3	74·3	376	206	30·2
0·001	127·3	75·2	377	208	41
0·0005	128·1	75·8	...	...	57
0·0002	128·8	76·4	...	...	80
0·0001	129·1	76·8	...	...	107

The cases quoted in the foregoing table are merely instances of the behaviour of aqueous solutions of electrolytes generally, and there is therefore no doubt that the efficiency of an electrolyte as a conductor of the electric current increases with dilution. The figures in the table supply the quantitative basis for the conclusion of which a qualitative demonstration has been described on p. 122. A study of the tabulated values for potassium chloride, sodium acetate, hydrochloric acid, and sodium hydroxide shows that λ is increasing only very slightly in the most dilute solutions, that, in fact, it tends towards a maximum value which could be found by extrapolating to zero concentration. This may be done on the basis of an empirical rule discovered by Kohlrausch, who showed that for most electrolytes in dilute solution there is a linear relationship between the equivalent conductivity and the cube root of the concentration. The value thus obtained by extrapolation is known as the equivalent conductivity at infinite dilution, and is indicated by the symbol λ_∞. Such an extrapolation can be safely made only in those cases in which λ changes but slightly in the most dilute solutions examined; it would, for instance, not

be permissible in the case of acetic acid, where λ is increasing rapidly even at the greatest dilutions. Where extrapolation is out of the question, another method of finding the value of λ_∞ must be adopted, a method that will be referred to later (p. 152). It should be noted that in this matter of extrapolation acetic acid is in quite a different category from sodium acetate, and the fact that the relative increase in λ between 1·0N and 0·0001N solutions is so much greater for acetic acid than for sodium acetate, is in harmony with the experiment described on p. 122.

What significance is to be attached to the values of λ_∞? According to the electrolytic dissociation hypothesis, a dissolved electrolyte takes part in the conduction of a current only in so far as it is ionised, and its efficiency in conducting the current will from this point of view be a maximum when ionisation is complete. The conducting efficiency, however, is, as we have seen, at a maximum in infinitely dilute solution, and therefore the value of λ_∞ for any electrolyte is to be taken as a measure of the total number of ions that can be produced by the dissociation of 1 gram-equivalent. Similarly, the value of λ at any finite dilution is a measure of the number of ions produced by the partial dissociation of 1 gram-equivalent of the electrolyte under these conditions. The extent to which the electrolyte is ionised, the degree of dissociation (a), is given therefore by the simple formula $a = \frac{\lambda}{\lambda_\infty}$.

The values of λ_∞ at 18° for potassium chloride, sodium acetate, hydrochloric acid, sodium hydroxide, and acetic acid are 129·9, 77·2, 383·3, 217·5, and 351·7 respectively. On the basis of these numbers and of the figures quoted in the table on p. 129, the following values of a have been calculated for a few selected concentrations:—

Gram-equivalents per litre.	KCl.	$CH_3.COONa$.	HCl.	NaOH.	$CH_3.COOH$.
1·0	0·76	0·53	0·79	0·73	0·004
0·5	0·79	0·64	0·85	0·79	0·006
0·1	0·86	0·79	0·91	0·84	0·013
0·01	0·94	0·91	0·96	0·92	0·041
0·001	0·98	0·97	0·98	0·96	0·117

This table shows very plainly that on the basis of the electrolytic dissociation hypothesis we must regard potassium chloride, sodium acetate, hydrochloric acid, and sodium hydroxide as being highly ionised in dilute solution, and a similar result would be reached by a consideration of the experimental data for all sodium and potassium salts of monobasic acids, for nitric acid and potassium hydroxide. Acetic acid, on the other hand, is only slightly ionised even in very dilute solution, and in this respect is typical of many monobasic organic acids, as well as of ammonia. There are however many acids which, as regards degree of dissociation, are intermediate between hydrochloric acid and acetic acid, just as there are many bases similarly intermediate between sodium hydroxide and ammonia.

Values of α Obtained by Different Methods.—Reference has already been made to the fact that the electrolytic dissociation hypothesis offers an explanation not only of the abnormal osmotic behaviour of acids, bases, and salts in aqueous solution, but also of the part which these compounds play in the conduction of an electric current. Our closer examination of the bearing of the hypothesis on these two classes of phenomena has shown that the degree of dissociation of a salt, acid, or base in aqueous solution can be estimated in two ways: (1) from the osmotic behaviour, specially from the freezing point, of the solution; and (2) from its conductivity. The vital question then arises: Are the values of α, based on determinations of the freezing point, in agreement with

those based on measurements of conductivity? The answer is, that although discrepancies occur in individual cases, the general parallelism between the two sets of values is so remarkable as to furnish a strong argument in support of Arrhenius's hypothesis. It was indeed this parallelism on which Arrhenius laid the main emphasis when the hypothesis was first brought forward. The general agreement between the values of a calculated from freezing point data and those derived from conductivity measurements is illustrated in the following table,[1] which embraces also certain figures for the osmotic activity of salts based on de Vries's isotonic coefficients. The last three columns of the table contain the values of i calculated (I.) from the depression of the freezing point; (II.) from the conductivity; (III.) from de Vries's figures.

Salt.	Gram-equivalents per litre.	I.	II.	III.
KCl . . .	0·14	1·82	1·86	1·81
$Ca(NO_3)_2$.	0·18	2·47	2·46	2·48
$MgSO_4$ . .	0·38	1·20	1·35	1·25
$CaCl_2$ . . .	0·184	2·67	2·42	2·78
K_4FeCy_6 . .	0·356	...	3·07	3·09

More recent and more accurate investigations have shown that the agreement between the values of a deduced from the freezing point and from the conductivity is in dilute solutions better than the foregoing table would indicate. This contention is supported by the following figures for potassium nitrate:—

Gram-equivalents per litre.	$i=\frac{t}{t_0}$ from Freezing Point.	$i=1+a$ from Conductivity.
0·02	1·90	1·91
0·025	1·87	1·89
0·05	1·84	1·87
0·10	1·79	1·83

[1] van't Hoff and Reicher. *Zeit. physikal. Chem.*, 1889, 3, 198.

It ought to be borne in mind that the values of a derived from freezing point experiments are valid for temperatures in the neighbourhood of 0° C., while those derived from electrical measurements are valid at 18° or 25°, at which temperatures most determinations of conductivity have been made. The degree of dissociation, however, does not alter much between 0° and 25°.

Utility of the Electrolytic Dissociation Hypothesis.—The evidence submitted so far shows that this hypothesis is capable of giving an intelligible interpretation of the abnormal depression of the freezing point on the one hand, and of the formation and behaviour of conducting solutions on the other hand. The remarkable parallelism between the values for the degree of dissociation deduced from the freezing points of salt solutions and those based on conductivity measurements creates a strong presumption in favour of the hypothesis, and it has therefore been widely adopted as a working theory of electrolytic solutions. Its utility in this respect cannot be denied, and although there are directions in which apparently the theory requires modification or extension, it has provided a satisfactory basis for the *quantitative* treatment of the phenomena exhibited by solutions of acids, bases, and salts. Evidence of the value of the theory from this practical standpoint will appear later.

It is perhaps desirable at this stage to emphasise once more the distinction which the theory makes between electrolytes and non-electrolytes. Arrhenius contends that the substances known as electrolytes are ionised in aqueous solution, and that in virtue of this ionisation their solutions conduct the electric current. The fact that a solution has a definite conductivity is evidence that the dissolved substance is ionised, and the conductivity

is taken as a measure of the ionisation. Non-electrolytes, on the other hand, are not ionised; they have a normal effect on the freezing point of water, and their solutions do not conduct the electric current. This broad distinction between electrolytes and non-electrolytes is not invalidated by the fact that there are many electrolytes which are close to the border line. Their aqueous solutions are very feeble conductors of the electric current, and their influence on the freezing point of water is nearly normal. This simply means that the degree of dissociation in such cases is extremely small. That there is, however, a fundamental distinction between a typical electrolyte, such as sodium chloride, and a typical non-electrolyte, such as sucrose, is clear from a consideration of their osmotic and electrical behaviour.

One objection which has been frequently urged against the electrolytic dissociation theory may be considered here, and that is the absence of a motive for dissociation. It is well known that the elements sodium and chlorine combine with extraordinary vigour to form sodium chloride, and that a very large amount of heat is developed when the combination takes place. Yet, according to the electrolytic dissociation theory, this compound is no sooner dissolved in water than the molecule is split up into two ions. This separation of the electrically charged atoms must obviously require a considerable amount of energy, and the question at once arises: From what source is this necessary energy derived? A full discussion of the question cannot be undertaken here, but it may be pointed out that much evidence has lately been accumulated showing that the ions are hydrated, that they carry about with them an envelope of water molecules. On the basis of this experimental material, the view has been brought forward that the attraction

of the ions for water is the real motive for dissociation in aqueous solution, and that the energy necessary for the separation of the ions is derived from the heat of their combination with water.[1]

[1] See Lowry, *Trans. Faraday Soc.*, 1905, **1**, 197; Bousfield and Lowry, *ibid.*, 1907, **3**, 123.

CHAPTER VIII

ELECTROLYTIC DISSOCIATION; PHYSICAL AND BIOLOGICAL APPLICATIONS

In the foregoing chapter the behaviour of acids, bases, and salts in aqueous solution has been contrasted with that of non-electrolytes, and it has been shown how the study of electrolytic solutions led up to the theory of ionic dissociation. The evidence discussed so far has been of a purely physical kind, but the theory has a highly important bearing on many physiological problems as well as on questions connected with the general behaviour of electrolytic solutions. As a preliminary, therefore, to a further consideration of the ionic hypothesis in its various aspects, it may be desirable to mention one or two facts which indicate the part played by electrolytes in the living organism.

The Conductivity of Physiological Fluids.—The fluids which bathe the tissues of plants and animals are electrolytic solutions. They contain, it is true, large quantities of non-electrolytic material, such as proteins, but they contain also appreciable quantities of salts, in virtue of which they are conducting fluids. Blood, for instance, is relatively a good conductor, the conductivity of the serum being nearly the same as that of a 0·7 per cent. sodium chloride solution. The figure found for the specific conductivity of ox blood serum at 25° varies between 0·0114 and 0·0131, and if the serum is diluted, the specific conductivity diminishes in the same way as that of an ordinary salt solution. If the quantity

of mixed salts in 1 litre of the undiluted serum is taken as a standard, and the conductivity of the diluted serum is in each case referred, not to 1 centimetre cube of solution but to this standard quantity of the mixed salts, numbers are obtained which are analogous to the equivalent conductivities recorded in the case of an ordinary salt solution, and which, like these, increase with dilution. By comparing the figure for the undiluted serum with the maximum figure obtained on dilution, it is possible to estimate the average degree of dissociation of the salts in the undiluted serum; this turns out to be from 0·65 to 0·76. The serum proteins, however, which amount to about 8 per cent., lower the conductivity of the undiluted serum more than that of the diluted serum, in which their concentration is much reduced, so that the foregoing figure is certainly too low. It is worth while noting by the way that the conductivity of defibrinated blood is only about half that of the corresponding serum. This is due to the fact that the defibrinated blood contains the corpuscles, which are non-conducting bodies, and diminish the conductivity by obstructing the active carriers of the current. The extent by which the conductivity of a sample of defibrinated blood is less than that of the corresponding serum has in fact been employed to calculate the total volume of the corpuscles in blood. The phenomenon is analogous to the lowering of the conductivity of a sodium chloride solution which results from the suspension of quartz powder in the solution.

Since blood and other physiological fluids are possessed of the characteristics of electrolytes, it is not surprising that the replacement of the fluids which normally bathe animal tissues by solutions of non-electrolytes should result in very marked modification of the activities of the tissues so treated. It has been found, for instance,

that if a frog muscle is allowed to lie in isotonic sucrose or dextrose solution long enough to extract all the salts from the fluid which bathes the muscle fibres, then the muscle gets into a condition in which it has no power either to transmit or respond to a stimulus; its contractility has disappeared. The power, however, is not destroyed; it is only rendered latent, for on the addition of sodium chloride or other sodium salts, the muscle is again able to respond to a stimulus.

While it is true that the greater part of the conductivity exhibited by physiological fluids is due to the presence of inorganic salts, yet there are other substances present which are partially ionised, and which therefore contribute to the conductivity of these fluids. Under the influence of enzymes changes take place in the organism, which result in the production of ionised from non-ionised substances. Proteins, for instance, are split up by the action of trypsin, an enzyme found in the pancreatic juice, and produce peptones and amino-acids, substances which are ionised to a certain extent. The course of such a protein degradation may therefore be followed by observing the increase of conductivity, or, what is the same thing, the decrease of resistance. The following figures supply an illustration of this phenomenon: they refer to the action of trypsin on a solution of caseinogen:[1]—

Time in Minutes.	Resistance in Ohms.
0	333·0
4	325·5
12	308·2
30	286·1
131	230·0
466	187·4
711	180·1

[1] Bayliss, *Journ. Physiol.*, 1908, 36, 221.

The decrease in viscosity which results from the action of trypsin in this case is quite inadequate to account for the increase in conductivity, and the latter must therefore be attributed to an increase in the number of current carriers, that is, the ions. The conductivity method of following the formation of ions which results from protein degradation has lately been employed in comparing the antiseptic value of disinfectants.[1]

The evidence quoted in the foregoing paragraphs may suffice to indicate in a preliminary way that in the processes associated with vital activity electrolytes must play no inconsiderable part. It is therefore desirable to consider the characteristic properties of electrolytic solutions in greater detail than we have as yet done, and to inquire how far the theory of ionic dissociation is capable of interpreting these properties adequately. One fact, for instance, which forces itself on all who study the behaviour of salt solutions is, that their properties are additive in character. What is the evidence for this generalisation, and supposing the evidence to be satisfactory, how is it to be explained?

The Additive Character of the Properties of Salt Solutions.[2] Evidence Based on their Chemical Behaviour.—It is generally recognised that the chemical reactions of a dissolved salt are simply the sum of the reactions which are characteristic of the positive part of the salt and those which are characteristic of the negative part. The behaviour of calcium chloride, for example, in dilute aqueous solution is not that of a compound which has its own individual peculiarities; the reactions of a dilute calcium chloride solution are simply those which are common to calcium salts *plus*

[1] Schryver and Lessing, *Journ. Soc. Chem. Ind.*, 1909, 28, 60.

[2] The phrase 'salt solutions' is to be understood as covering solutions of acids and bases.

those which are common to chlorides. The significance of this is apparent, in view of the fact that in a chemical compound the characteristics of the components cannot as a rule be detected; the properties of a given element are modified to an extent which depends on the other element or elements with which it has combined. Sulphur, for instance, unites both with carbon and with oxygen, forming carbon disulphide and sulphur dioxide respectively, but it is quite impossible to regard the properties of these two compounds as the sum of the properties of the components; the characteristics of sulphur, which would in that case be exhibited by both compounds alike, are conspicuously absent.

The additive character of the reactions of dilute salt solutions is emphasised by contrast with the behaviour of organic substances. The existence of a common atom or group of atoms in these substances cannot be proved by the simple precipitation reactions on which we rely for the recognition, say, of bromides or sulphates in aqueous solution. The reactions of an organic compound, even in solution, are as a rule not resolvable into the reactions of the component atoms or groups. For instance, an aqueous solution of potassium ethyl sulphate is not precipitated by the addition of barium chloride, and alcoholic solutions of silver nitrate and phenyl bromide may be mixed without giving any precipitate of silver bromide.

The electrolytic dissociation hypothesis supplies an interpretation of the additive character of reactions in salt solutions. According to this hypothesis, dilute solutions of sulphuric acid, copper sulphate, and potassium sulphate are alike in this, that they all contain large quantities of the $\overline{SO}_4$ ion, so that when barium chloride is added to each of these solutions the same result

follows. It is possible however for a compound containing the $-SO_4$ group, such as potassium ethyl sulphate, to dissolve without being ionised, or to ionise in a different way from ordinary sulphates, and in such a case the addition of barium chloride may not cause any precipitation whatsoever. Similarly, the failure of silver nitrate to precipitate phenyl bromide in alcoholic solution is to be attributed to the non-ionisation of phenyl bromide. From the point of view, then, of the electrolytic dissociation theory, the reactions which are so largely employed in analytical chemistry are ionic reactions, and the behaviour of a salt in dilute solution may be regarded as the reactions of the positive ion *plus* those of the negative ion. The observation that a compound which is very reactive in dilute aqueous solution frequently loses this character when dissolved in a non-ionising solvent is instructive in this connection. Thus acids in aqueous solution are characterised by their power of acting on carbonates, and yet a solution of dry hydrogen chloride in benzene —a solution, it should be observed, which does not conduct the electric current—is unable to attack dry sodium carbonate.[1] Since this solution is a non-conductor, we may conclude that the dissolved hydrogen chloride is in the un-dissociated or un-ionised condition; it appears, therefore, that the reactions of hydrogen chloride in aqueous solution are quite different from its reactions in the un-ionised condition. It has sometimes been suggested that all instantaneous reactions, such as those occurring in the precipitation of one salt by another, are ionic reactions, but this statement is too sweeping. Kahlenberg[2] has found cases of double decomposition accompanied by immediate precipitation

[1] See Kahlenberg, *Journ. Physical Chem.*, 1902, 6, 1.

[2] *Loc. cit.*

in solutions which are excellent insulators. Thus a solution of dry hydrogen chloride in benzene and a solution of dry ammonia in benzene are both non-conductors like benzene itself, and yet, when mixed, they give instantly a white precipitate of ammonium chloride.

The Colour of Salt Solutions.—If we take a series of coloured salts the colour of which springs from the presence of a particular metal or a particular acid radical, it is found that dilute solutions of the salts of each series have all the same colour. This is the case even when the solid salts or their concentrated aqueous solutions differ in colour; any such difference tends to disappear with dilution. Concentrated cupric chloride solutions are green, and in this respect differ from concentrated copper sulphate solutions, which are blue; the green solutions, however, turn blue on dilution, and are then indistinguishable, so far as the colour goes, from dilute copper sulphate solutions. The colour of a cupric salt in dilute solution is in fact independent of the acid radical, provided that the latter itself makes no contribution to the colour. The additive character of the colour of a salt in dilute aqueous solution is brought out very clearly by a study of absorption spectra. Ostwald has recorded photographically [1] the absorption spectra of solutions of the permanganates of lithium, cadmium, ammonium, zinc, potassium, nickel, magnesium, copper, hydrogen, aluminium, sodium, barium, and cobalt (in all cases 0·002 gram-equivalent per litre). The absorption bands are practically identical for all these solutions, and occupy the same positions in the spectrum. This striking result strongly supports the contention that the colour of a dilute salt solution

[1] *Zeit. physikal. Chem.*, 1892, 9, 579

is an additive property based on the independent contributions made by the metallic and acidic parts of the salt. An intelligible explanation of this independence of the metallic and acidic parts of a salt is furnished by the electrolytic dissociation theory, according to which a dilute salt solution is mainly a mixture, in electrically equivalent quantities, of the two ions. The theory postulates that the spheres of influence of these ions are distinct, and that in regard to colour as well as chemical reactivity, each ion makes its characteristic contribution to the properties of the solution.

Ionic Conductivity.—Evidence of a more definitely quantitative kind in favour of the view that the metallic and acidic parts of a salt are to a large extent independent of each other in dilute solution is obtained by considering the way in which the value of the equivalent conductivity varies from one salt to another. Suppose that for this purpose we deal with the figures recorded in the following table; they represent the equivalent conductivities found for half-a-dozen alkali salts at 18° in 0·0001 normal concentration:[1]—

	Chloride.	Nitrate.
Potassium	129·05	125·49
Sodium	108·06	104·53
Lithium	98·06	94·38

A glance at these figures will show that $\lambda_{KCl} - \lambda_{NaCl} = 20{\cdot}99$, and that $\lambda_{KNO_3} - \lambda_{NaNO_3} = 20{\cdot}96$, practically the same figure. Further, $\lambda_{KCl} - \lambda_{LiCl} = 30{\cdot}99$, while $\lambda_{KNO_3} - \lambda_{LiNO_3} = 31{\cdot}11$, practically the same figure. Again, $\lambda_{KCl} - \lambda_{KNO_3} = 3{\cdot}56$, $\lambda_{NaCl} - \lambda_{NaNO_3} = 3{\cdot}53$, and $\lambda_{LiCl} - \lambda_{LiNO_3} = 3{\cdot}68$. Expressed in words, these figures mean that the change in the value of the equivalent conductivity produced

[1] Kohlrausch and Maltby, *Sitzungsber. k. Akad. Wiss. Berlin*, 1899, 665.

by substituting a sodium salt or a lithium salt for a potassium salt is the same whether the salt is a chloride or a nitrate; that is, the metallic part of the salt makes a contribution to the conductivity which is independent of the acidic radical with which it is associated. The values of the last three differences show similarly that the substitution of a nitrate for a chloride of equal concentration leads to a decrease of λ, which is the same whether the metallic part of the salt is potassium, sodium, or lithium. Similar relationships would be found to exist if we dealt with the values of λ_∞, obtained by extrapolation, instead of the values of λ for 0·0001 normal solutions, and we may therefore conclude that the contribution which an ion makes to the equivalent conductivity of a highly diluted solution is independent of the other ion with which it is associated. Kohlrausch, who first detected the additive character of the conductivity of a highly diluted salt solution, expresses the independence of the ions in this respect by the equation $\lambda_\infty = u + v$, where u and v are the contributions which the cation and anion respectively make to the equivalent conductivity at infinite dilution. This equation is the expression of what is generally known as Kohlrausch's Law of the Independent Migration of the Ions, and the terms u and v which appear in the equation are described as ionic conductivities. The value of u for a given cation remains the same for all salts which contain this cation, just as the value of v for a given anion remains the same whatever be the salt of which it forms part. The actual numerical values of u and v cannot however be obtained until some other equation is available which involves these quantities.

The numbers recorded in the last table show very clearly that the contribution made to the conductivity by the lithium ion is less than that made by the sodium

ion, and this again is less than the contribution made by the potassium ion. In view of this the question at once suggests itself: Why should one ion contribute more than another to the conductivity of a solution? If, in accordance with the theory of electrolytic dissociation, we conceive the passage of a current through an electrolyte as consisting in the movement of electrically charged material particles, we might regard the superior efficiency of a given ion in the conduction of the current as due either to its carrying a greater charge, or to its moving more rapidly than other ions under the same conditions. The first explanation cannot be maintained, for Faraday has shown that the quantities of different ions liberated during electrolysis by a given current are in the ratio of their chemical equivalents; that is, with a gram-equivalent of each ion there is associated the same definite quantity of electricity. All univalent ions—for example, $K^{\cdot}$, $Na^{\cdot}$, Cl', NO_3', $NH_4^{\cdot}$—must therefore carry the same charge. We are driven accordingly to the second possible explanation of the difference in the contributions made by various ions to the conductivity, namely, that, exposed to the same electrical forces, different ions have different mobilities: one ion may be faster or slower than another ion. The acceptance of this view involves certain conclusions as to changes of concentration which must accompany the process of electrolysis. We shall first deduce these conclusions, and then compare them with the results of experimental work.

The assumption that the contribution which an ion makes to the equivalent conductivity depends on its mobility may be expressed more definitely by the equation $\frac{u}{v} = \frac{\text{speed of cation}}{\text{speed of anion}}$, and it is easy to show that if the ions of a salt move at different rates, the fall of con-

centration round the anode due to electrolysis is different from the fall of concentration round the cathode. Suppose that the condition of an electrolytic solution before electrolysis commences is represented diagrammatically, as in Fig. 17. Between the anode A and the cathode C there is, we may suppose, only a limited number of fully ionised molecules. The electrolytic

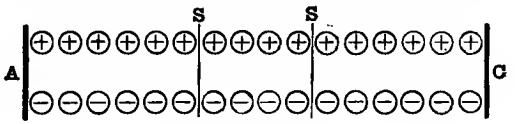

FIG. 17.

cell may be conceived as divided into three parts, a compartment round the anode and one round the cathode, each containing six fully ionised molecules, as well as an intermediate compartment containing four fully ionised molecules. The compartments are separated from one another by the porous septa SS.

Suppose, to begin with, that the positive and negative ions move at the same rate. If a current is passed just so long that two cations cross each of the septa SS from left to right, then in this time two anions will have crossed the septa from right to left, and the position of matters will be as represented in Fig. 18. In the

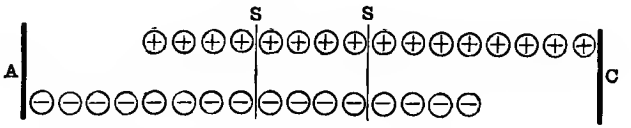

FIG. 18.

intermediate compartment there will still be four molecules as before the electrolysis; the concentration there is unaltered. The isolated ions are those which have been liberated at the electrodes during the passage of

the current: the number of these liberated ions is the same at each electrode, as required by Faraday's law. In the solution round the anode there are now four molecules—a loss of two molecules; in the cathode compartment there are four molecules left—likewise a loss of two molecules. Hence, when the ions move at the same rate, the fall of concentration round the anode is equal to the fall of concentration round the cathode. Suppose next that the speed of the cation is twice as great as that of the anion, and that the current passes just so long that two cations pass across each of the septa SS_1 from left to right; in this time one anion will pass across each septum from right to left, and the position of matters will then be as represented in Fig. 19.

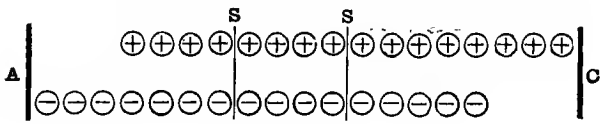

FIG. 19.

As before, the concentration in the intermediate compartment is unaltered, while three ions have been liberated at each electrode. The number of molecules left in the anode compartment is now four—a loss of two; the number of molecules left in the cathode compartment is five—a loss of one. We have therefore

$$\frac{\text{Fall of concentration round anode}}{\text{Fall of concentration round cathode}} = \frac{2}{1} = \frac{\text{speed of cation}}{\text{speed of anion}}.$$

This line of argument might be extended to cover other speed ratios, and a similar conclusion would be reached. On the basis, therefore, of the view that different ions make contributions to the equivalent conductivity in proportion to their rates of migration

under the action of the same electrical force, we have $\frac{u}{v} = \frac{\text{speed of cation}}{\text{speed of anion}} = \frac{\text{fall of concentration round anode}}{\text{fall of concentration round cathode}}$, provided that in any experiment carried out in order to determine the relative speed of the ions there is an intermediate zone of the electrolyte in which no change of concentration has taken place. If we adopt the view that during the electrolysis of a salt solution the ions are moving at different speeds, then it is obvious that of the electricity which is transported across any given section of the electrolyte, a greater fraction will be carried by one ion than by the other. If, for instance, the cation moves twice as fast as the anion, then two cations will cross a given section of the electrolyte from left to right, while one anion is crossing the same section from right to left; since the ions carry equal charges, this means that the quantity of positive electricity transported across the section is twice as great as the quantity of negative electricity. To put it generally, let us suppose that of the total transported electricity the fraction n is carried by the anions and the fraction $1-n$ by the cations; then

$$\frac{1-n}{n} = \frac{u}{v} = \frac{\text{fall of concentration at anode}}{\text{fall of concentration at cathode}}.$$

Now there is a well-known algebraic theorem which states that if $\frac{a}{b} = \frac{c}{d}$, then $\frac{a}{a+b} = \frac{c}{c+d}$; if this theorem is applied to the foregoing equations, it is easily shown that

$$1-n = \frac{u}{u+v} = \frac{\text{fall of concentration at anode}}{\text{total fall of concentration}},$$

and

$$n = \frac{v}{u+v} = \frac{\text{fall of concentration at cathode}}{\text{total fall of concentration}}.$$

Hittorf's Work.—So far we have simply attempted to deduce the conclusions that follow from the assumption of different ionic velocities, and we may now ask

Do changes of concentration occur round the electrodes during electrolysis, and if so, is the fall of concentration at one electrode in some cases different from that at the other electrode? These questions were answered long ago in the affirmative by the classical work of Hittorf, who determined the values of n and $1-n$, the transport or migration numbers as they are called, for various anions and cations. A particular example may perhaps be quoted to show the sort of experimental data which Hittorf obtained, and the way in which he employed these data to calculate the transport number. An electrolytic cell containing a solution of copper sulphate was put in series with one containing a solution of silver nitrate. After a current had been passed for some time, it was found that 1·008 gram of silver had been deposited on the cathode of the silver nitrate cell. According to Faraday's law, this amount of silver must be equivalent to the copper deposited on the cathode of the copper sulphate cell; this weight of copper must therefore be $1{\cdot}008 \times \frac{31{\cdot}8}{108} = 0{\cdot}2968$ gram, a figure which is a measure, in terms of copper, of the total loss of concentration in the copper sulphate cell. Before electrolysis the solution round the cathode contained, as shown by analysis, an amount of copper sulphate equivalent to 2·8543 grams of copper oxide: after electrolysis the cathode solution gave on analysis 2·5897 grams of copper oxide. Electrolysis has resulted therefore in a fall of concentration at the cathode represented by 0·2646 gram CuO or 0·2114 gram Cu. This loss, however, is less than the weight of copper which has been deposited on the cathode out of the surrounding solution, namely, 0·2968 gram, and it is therefore obvious that the difference, $0{\cdot}2968 - 0{\cdot}2114 = 0{\cdot}0854$ gram, must have migrated from the anode com-

partment into the cathode compartment. The figure 0·0854 represents, in terms of copper, the fall of concentration round the anode, and we have accordingly $1-n=\frac{\text{fall of concentration at anode}}{\text{total fall of concentration}}=\frac{0\cdot0854}{0\cdot2968}=0\cdot288$, which is therefore the transport number for the copper ion in this solution. The transport number for the sulphate ion is 0·712, and a comparison of these figures shows that of the total electricity transported across any section of the electrolyte about seven-tenths is carried by the negative ions.

Numerical Values for Ionic Conductivity.—From the work of Hittorf and others who have followed him, we know then the ratio of the contributions which the ions of an electrolyte make to the equivalent conductivity. The value of this ratio may not be the same in concentrated and in dilute solutions of the electrolyte, but it is found on investigation that after a certain stage of dilution no further change in the value of the ratio takes place. As an illustration of this we may take the following figures obtained by Hittorf for the transport number $(1-n)$ of silver in silver nitrate solutions of different concentration :—

Weight of Water to 1 gram $AgNO_3$.	$1-n$.
2·48	0·532
2·73	0·522
5·18	0·505
10·38	0·490
14·5	0·475
49·4	0·474
247·3	0·476

These figures show that the transport number for silver in dilute solutions is 0·475, and that this value does not alter over a considerable range of concentration.

So for other ions values of the transport numbers are obtained which are valid for highly diluted solutions, and which can be used in the following way to calculate ionic conductivities. We have seen that $\lambda_\infty = u + v$, $1 - n = \frac{u}{u+v}$, and $n = \frac{v}{u+v}$; hence it follows that $u = (1-n)\lambda_\infty$, and $v = n\lambda_\infty$. The value of λ_∞ for a salt is ascertained, as already shown, by extrapolating from the actually observed figures for λ, while the values of n and $1-n$ are given by Hittorf's work. As an example of the way in which ionic conductivities are calculated, the case of potassium chloride may be taken. For this salt λ_∞ at $18° = 129{\cdot}9$, while the transport number for chlorine is $0{\cdot}503$. We have then $u = 0{\cdot}497 \times 129{\cdot}9 = 64{\cdot}6$, and $v = 0{\cdot}503 \times 129{\cdot}9 = 65{\cdot}3$; that is, the ionic conductivity of potassium at 18° is 64·6, and the ionic conductivity of chlorine is 65·3 at the same temperature.

In a similar manner, by combining the values of n, $1-n$, and λ_∞ for any electrolyte it is possible to calculate other ionic conductivities. It is noteworthy, however, that when one ionic conductivity has been evaluated, all others can be calculated from it by means of the formula $\lambda_\infty = u + v$, without any further determination of transport numbers. Suppose, for instance, that on the basis of the value $0{\cdot}503$ for the transport number of chlorine in potassium chloride the ionic conductivity of chlorine at 18° has been found to be 65·3, as just shown. Then since λ_∞ for sodium chloride at 18° has been found to be 108·8, and since, according to Kohlrausch's law of the independent migration of the ions, λ_∞ for $NaCl = u_{Na} + v_{Cl}$, we have $108{\cdot}8 = u_{Na} + 65{\cdot}3$, whence $u_{Na} = 43{\cdot}5$.

The following table records the values of the conductivity at 18° for various ions:—

Cations.		Anions.	
H	318	OH	174
Li	33·4	Cl	65·3
Na	43·5	I	66·4
K	64·6	NO_3	61·8
NH_4	64·4	$CH_3.COO$	33·7
Ag	54·0		

These figures, it should be noted, are based on the investigation of electrolytes for which λ_∞ can be determined by extrapolation from the measured values of λ. So soon, however, as the values of u and v have been ascertained for various ions it becomes possible to calculate the value of λ_∞ for electrolytes where an extrapolation cannot be made. Acetic acid supplies an instance of this. A glance at the figures for acetic acid recorded in the table on p. 129 shows that even at the highest dilutions the value of λ is still increasing so rapidly that an extrapolation is not permissible. But if Kohlrausch's law is valid for acetic acid at infinite dilution as it is for other electrolytes, then $\lambda_\infty = u_H + v_{Ac}$, where u_H is the ionic conductivity of hydrogen, and v_{Ac} is the ionic conductivity of the acetate radical. The values of u_H and v_{Ac} have been ascertained by a study of strong acids and of alkali acetates, and are recorded in the table of ionic conductivities. Hence for acetic acid $\lambda_\infty = 318 + 33{\cdot}7 = 351{\cdot}7$, a figure which has been quoted already on p. 130.

Actual Velocity of Migration of the Ions.—The method employed in deducing the values of the ionic conductivities is based on the view that electrolysis consists in a streaming of positively charged ions in one direction and of negatively charged ions in the opposite direction, that the positive and negative ions may move at different rates, and that to this cause is due the difference in the contributions which the two ions of an electrolyte make to the equivalent conductivity. This view is con-

firmed by the concentration changes which do occur during electrolysis, and by the relative magnitude of these changes round anode and cathode respectively. The values of u and v already quoted give, however, no direct information as to the actual speed at which the ions move under the action of a given electromotive force. They are measured in the same units as the equivalent conductivity, and enable us in the first place to deduce only the *relative* speeds of the two ions of an electrolyte under the same conditions. The *actual* speed of any particular ion will of course depend on the magnitude of the electric force which is acting on it, in other words, on the steepness of the potential gradient between the two electrodes.

It is, however, possible to calculate from the ascertained values of ionic conductivity the actual rates at which the ions move when the fall of potential through the electrolyte has some definite value, say 1 volt per cm. The details of this calculation cannot be given here, but the results may be illustrated by the following figures. Provided that the fall of potential in the electrolyte is 1 volt per cm., the hydrogen ion moves at the rate of 0·0033 cm. per second, the hydroxyl ion 0·0018 cm. per second, and the potassium ion 0·00067 cm. per second. If in some particular case the fall of potential were 10 volts per cm., then the rates at which the ions move would be ten times as great.

Not only is it possible to calculate the actual velocity of the ions; it can be determined by direct observation. The way in which this is possible is illustrated by the following experiment, first suggested by Nernst. A glass tube, about 1 mm. bore, is sealed at one end to a small tap funnel and at the other to a **U** tube, each limb of which is 5–8 mm. diameter. The capillary tube is then bent as shown in Fig. 20. A dilute solution of potassium permanganate (0·003 normal relatively to

potassium), to which 5–10 per cent. of urea has been added in order to increase its density, is poured into the funnel, and the tap is opened until the capillary tube is filled as far as its junction with the U tube. The tap is then closed, and the U tube is half or two-thirds filled with a 0·003 normal solution of potassium nitrate. The stopcock is again carefully turned on, and the permanganate solution is allowed to occupy the bottom of the U tube slowly, pushing the potassium nitrate solution before it into each limb. When the U tube is completely full the stopcock is finally turned off. We thus obtain a column of potassium permanganate solution isolated between two columns of potassium nitrate solution. Two platinum wires connected with the terminals of a powerful battery, or say a 100-volt lighting circuit, are dipped in the solution at the top of each limb, the positive wire being placed in the right-hand limb. After the current has been running for a short time it is seen that the boundary between coloured and colourless solution is higher in the right limb than in the left; that is, the permanganate ion which is responsible for the colour of the permanganate solution has visibly advanced towards the anode. If the advance of the boundary is measured, and if the potential difference between the electrodes, as well as their distance apart, is known, we may estimate the actual rate at which the permanganate ion would migrate if the fall of potential were 1 volt per cm.

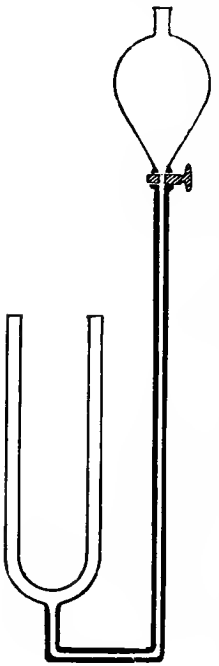

FIG. 20.

The correctness of this estimate depends on whether the fall of potential is regular throughout the whole column of electrolyte between the electrodes. This would be the case only if the specific conductivity of the permanganate solution were the same as that of the potassium nitrate solution. It is evident, therefore, that any determination of the rate at which an ion moves involves a knowledge not only of the distance covered, but also of the exact potential gradient. A discussion of the means adopted to ascertain the potential gradient, and of the conditions necessary to secure a sharp boundary between two solutions during electrolysis, is beyond the scope of this volume, but it may be mentioned that the advance of a boundary even between two colourless solutions can be followed by a method depending on the difference in refractive index.[1]

Ionic Conductivity and Hydration.—Consideration of the numerical values of the ionic conductivity raises a point of great interest in connection with the theory of solutions. In the group of alkali metals, as recorded on p. 152, $u_{Li} = 33{\cdot}4$, $u_{Na} = 43{\cdot}5$, and $u_{K} = 64{\cdot}6$; the lightest metal furnishes, therefore, the most sluggish ion of the three, and the heaviest metal yields the most speedy ion. This curious result is now generally attributed to the different hydration of the ions.[2] It is supposed that of the three the lithium ion is hydrated to the greatest extent, and that the size of the water 'envelope,' of which the lithium ion is the nucleus, is responsible for the greater friction experienced by it in passing through the water, and therefore for its smaller mobility. The potassium ion, on the other hand, is pre-

[1] Steele, *Journ. Chem. Soc.*, 1901, 79, 414.

[2] See Kohlrausch, *Proc. Roy. Soc.*, 1903, 71, 338; Bousfield, *Proc. Roy. Soc.*, 1905, 74, 563; *Phil. Trans.*, A, 1906, 206, 101; Senter, *Science Progress*, Jan. 1907.

sumably hydrated to a less extent than either the sodium or the lithium ion. It is further noteworthy that for the three ions already mentioned the temperature coefficient of the mobility is greatest for the lithium ion and smallest for the potassium ion. This observation is at least in harmony with the view that the relative hydration is as suggested, for rise of temperature is bound to favour the breaking down of the hydrates, and the effect of a rise of temperature would probably be most marked in the case of the ion which is most highly hydrated.

The view that the ions of an electrolyte are hydrated finds support in the observation that the temperature coefficient of the conductivity of a dilute solution is practically the same as the temperature coefficient of the fluidity of water $\left(\text{fluidity} = \frac{1}{\text{viscosity}}\right)$. This seems to show that the resistance which the ions experience in their movements is the frictional resistance of the solvent, a result which becomes intelligible if it is supposed that each ion carries a water envelope along with it.

Ionic Conductivity and the Diffusion of Electrolytes.—The difference in the contributions which various ions make to the equivalent conductivity of an electrolyte has been attributed to the difference in their speeds. The figures recorded in the table on p. 152 are therefore a measure of the speeds at which the ions move under the action of a given electromotive force; they are proportional to the 'mobility' of the ions. Now the mobility of an ion will come into play not only when it is in an electric field, but when it is involved in a concentration gradient, that is, when the salt of which it forms part is diffusing from places of high concentration to places of low concentration.

In the case of hydrochloric acid, for instance, the fact that the mobility of the hydrogen ion is about five times that of the chlorine ion must have a direct bearing on the rate of diffusion.

Suppose that a solution of hydrochloric acid is in contact with pure water. Diffusion occurs, and it might be thought in view of their relative mobilities that the hydrogen ions would soon outstrip the slower chlorine ions. A little reflection, however, shows that such a separation of ions cannot take place except to an infinitesimal extent. In consequence of the greater mobility of hydrogen, the front rank of the diffusing acid will consist of positive hydrogen ions, while behind these there will be an excess of negative ions. Electrostatic forces are thus called into action, which prevent anything more than an infinitesimal separation, and which have the effect of retarding the advance of the hydrogen ions and accelerating that of the chlorine ions. The net result is that the acid diffuses as a whole without any measurable separation of hydrogen and chlorine, the different natural mobilities of the two ions being compensated by the action of the electrostatic forces. It is evident, however, that the rates of diffusion of different chlorides will depend on the mobility of the positive ion, on its ability to push on in front and so accelerate the advance of the chlorine ion. We may therefore expect that when various chlorides are arranged in order according to their rates of diffusion in aqueous solution, the order will be the same as that of the conductivities of the positive ions; similarly, we may expect that when various sodium salts are arranged according to their rates of diffusion in aqueous solution, the order will be the same as that of the conductivities of the negative ions. These expectations are borne out by the figures in the following tables, which give

the diffusion coefficients of various chlorides and various sodium salts at 18° :—

	Diffusion Coefficient.		Diffusion Coefficient.
HCl . . .	2·30	NaOH . . .	1·40
KCl . . .	1·46	NaCl . . .	1·14
NaCl . . .	1·14	$NaNO_3$ . . .	1·03
LiCl . . .	1·00	$NaCH_3COO$.	0·78

Reference to the table of ionic conductivities on p. 152 will show that in regard to mobility, $H^{\cdot} > K^{\cdot} > Na^{\cdot} > Li^{\cdot}$, and that $OH' > Cl' > NO_3' > CH_3COO'$.

The difference in mobility of various ions, then, is modified, so far as diffusion is concerned, by the electrostatic attraction between the ions, and gives rise to a difference of potential at the common surface (1) of salt solution and water, (2) of differently concentrated solutions of the same salt, or (3) of solutions of different salts. It is in this direction that we must seek for an explanation of the electrical effects which, as found by physiologists, so frequently accompany vital activity. Differences of electrical potential in the tissues are probably due to a separation (infinitesimal in extent) of the positive and negative ions of the electrolytes which bathe these tissues.

It will be apparent from the foregoing that the positive and negative ions of an electrolyte are not absolutely independent. The charges which the ions carry are responsible for the intervention of electrostatic forces, and these limit the independence of the ions, so far at least as their separation is concerned. Another case in which the factor of electrostatic attraction between the ions has a definite bearing is the problem of the permeability of living membranes to electrolytes. So far as reference has been made to this problem in the present volume, the behaviour of salts

only as indivisible units has been considered. We have however now adopted the view that salts are more or less ionised in aqueous solution, and that the ions are in many respects independent of each other. The questions then naturally arise: Is it not possible that the two ions of a salt are characterised by a different power of penetrating the living membrane? If so, what would be the result if a solution of the salt were separated from pure water by such a membrane? If we assume for the moment that the ions of a salt do differ in their power of penetration, and, taking an extreme case, we suppose that the membrane is permeable to the anion but impermeable to the cation, then a little consideration shows that the salt as a whole cannot penetrate the membrane. For the passage of the anions through the membrane would mean a separation of the ions; this, as has been already shown, is opposed by the electrostatic forces, and can take place to an extent which, so far as analytical methods of detection go, is absolutely negligible; the membrane would be practically impermeable to the salt. It would, however, be the seat of a potential difference originating in the same manner as the potential difference at the common surface of salt solution and water, and the possibility of electrical effects arising in this way at the surface of a membrane bathed by an electrolyte has an important bearing on the problems of electro-physiology.[1]

In the case of the salt just described the anions are prevented from passing through the membrane by the inability of their positive partners. Actual transport of these anions through the membrane would be rendered possible however either (1) by adding to the salt solution another electrolyte the cation of which is able to penetrate the membrane, or (2) by adding to the water on the further side a salt for the anion of which the

[1] See Donnan, *Zeit. Elektrochem.*, 1911, 17, 572.

membrane is permeable. In the first case, the cation of the added salt and the anion of the original salt could cross the membrane together in electrically equivalent quantities; in the second case, there would be an exchange of the two anions, also in electrically equivalent quantities.

This is not an imaginary picture, for investigations by Hamburger, Köppe, and others[1] have shown that the plasmatic membrane of blood corpuscles is generally permeable to anions. Some of the facts which support this conclusion may be quoted briefly. When a current of carbon dioxide is passed through blood, chlorine passes from the serum into the corpuscles, and the alkalinity of the serum increases. Again, if blood corpuscles are separated by centrifuging, suspended in an isotonic solution of a neutral sodium salt and subjected to a current of carbon dioxide, the salt solution becomes strongly alkaline. If, on the other hand, the separated corpuscles are suspended in an isotonic solution of sucrose or dextrose and there subjected to a current of carbon dioxide, no alkalinity results. The most satisfactory explanation of these phenomena is based on the view that the carbon dioxide penetrates the covering of the blood corpuscles, and reacting with some of the corpuscle contents, probably the proteins, gives rise to the carbonate ions HCO_3' and CO_3''. The plasmatic membrane being permeable to anions, an exchange between these carbonate ions and chlorine ions in the surrounding fluid becomes possible, and leads to the production of sodium carbonate, and consequent alkalinity, in the sodium salt solution.

Emphasis has already been laid on the condition that any such exchange of ions across a membrane

[1] Hamburger, *Zeitsch. Biol.*, 1891, 28, 405; v. Limbeck, *Arch. exper. Pathol.*, 1895, 35, 309; Köppe, *Pflüger's Arch.*, 1897, 67, 189; Hamburger and van Lier, *Engelmann's Arch. Physiol.*, 1902, 492.

must take place in electrically equivalent proportions. If in the case of blood corpuscles the CO_3'' ion is exchanging with the Cl' ion, it is obvious that for every carbonate ion that leaves a corpuscle two chlorine ions must enter; in order to preserve osmotic equilibrium between the corpuscle contents and the surrounding solution, water also must pass in, and the bulk of the corpuscles must increase. No such increase in the volume of the corpuscles is to be expected if the CO_3'' ion is exchanging with the SO_4'' ion. The correctness of this line of argument has been confirmed by experiment in the following way. Equal quantities of blood corpuscles are suspended in isotonic solutions of (1) sucrose, (2) sodium sulphate, (3) sodium chloride, (4) sodium nitrate, (5) potassium nitrate, and a current of carbon dioxide is passed in each case. The volumes occupied by the corpuscles after centrifuging are equal for cases (1) and (2), equal also for cases (3), (4), and (5), but greater for the second set than for the first.

From recent investigations it appears that the covering of red blood corpuscles is permeable not only for anions, but also, in certain cases at least, for cations. According to Hamburger,[1] when a small quantity of calcium chloride is added to bullock's blood, the calcium distributes itself between serum and corpuscles, that is, the covering of the corpuscles is permeable to the calcium ion. Such corpuscles, further, into which calcium has thus penetrated, lose the extra amount they have taken up when they are washed with normal serum; the calcium ion, that is, can pass out as well as in. Hamburger maintains that the passage of calcium ions into the corpuscles occurs only when an exchange with other cations is possible.

[1] *Zeit. physikal. Chem.*, 1909, 69, 663.

If it should turn out on further investigation that the red blood corpuscles are permeable for cations generally, then Overton's generalisation, according to which the permeability relationships of plant and animal cell membranes are alike (see p. 80), would have to be modified.

Specific Action of Ions.—In the foregoing paragraphs attention has been drawn to a property possessed by only one ion of an electrolyte, the manifestation of which is restricted by the action of electrostatic forces. There are, however, other properties which are specifically characteristic of either the anion or the cation, and the manifestation of which is free from any such limitation. That we should be able to detect the specific activity of any one ion is only natural in view of the generally additive character of the properties of electrolytes, and is further in harmony with the comparative independence of the ions postulated by the electrolytic dissociation theory.

The influence of various alkali salts on the contractility of muscle may be taken as an instance of the way in which a specific property is associated with some particular ion or ions. On p. 138 it was stated that if a frog muscle is allowed to lie in isotonic sucrose or dextrose solution long enough to extract all the salts from the fluid which bathes the muscle fibres, the muscle loses its power of transmitting or responding to a stimulus. The contractility, however, is restored on treatment of the muscle with solutions of sodium salts. Any sodium salt serves for this purpose; the character of the anion with which the sodium is associated is practically immaterial.[1] This observation in the physiological field is closely related to the fact, already discussed, that the chemical reactions of salt solutions are additive in character; the solutions of calcium salts,

[1] Overton, *Pflüger's Archiv.*, 1904, 105, 176.

for instance, give certain reactions which are the same whether it is the nitrate, the chloride, or the sulphate which is employed; the character of the anion with which the calcium is associated is immaterial. In contrast to the power of sodium salts to restore the contractility of muscle stands the behaviour of potassium salts; none of these is able to neutralise the paralysing effect of treatment with sucrose solution. Further investigation on these lines shows that the sodium salts are in a category by themselves, and that the maintenance of contractility is a specific function of the sodium ion.

In this or any other case where some effect is specifically associated with the one ion of an electrolyte as distinct from the other ion and from the undissociated molecule, then the magnitude of the effect ought manifestly to depend on the degree of the ionisation. This conclusion has been verified to some extent by the work of Paul and Krönig[1] on the germicidal effect of various salts. For the purpose of comparison the salt solutions were allowed to act for a given time on approximately equal numbers of anthrax spores, and the number of colonies which developed subsequently was taken as a measure of the germicidal power of the salt solution. Other conditions being kept uniform, it was found that the number of colonies which develop after treatment of the spores with a given salt decreases as the treatment is prolonged and as the concentration of the salt solution is increased. For equally concentrated solutions of salts, all containing a cation of marked germicidal power, the number of colonies developed ought to increase as the degree of dissociation diminishes. Paul and Krönig tested this contention by comparing the disinfecting power of mercuric chloride, bromide, and

[1] *Zeit. physikal. Chem.*, 1896, 21, 414.

cyanide; it is known that for equal concentrations of these salts the degree of dissociation is greatest in the case of the chloride, and least in the case of the cyanide. The following table gives the results obtained:—

Disinfecting Solution.	Number of Colonies developed after Treatment lasting for	
	20 Minutes.	85 Minutes.
$HgCl_2$ (1 mol. in 64 litres)	7	0
$HgBr_2$ („ „ „)	34	0
$Hg(CN)_2$ („ 16 „)	∞	33

On the assumption that the germicidal effect of the undissociated molecules and of the anions is negligible, these figures are in harmony with the view that the degree of dissociation of the three mercury salts increases from the cyanide to the chloride. So far therefore we may lay down the proposition that the disinfecting power depends not on the total concentration of mercury salt, but on the concentration of the mercuric ion.[1] But when mercury salts other than the halogen salts are investigated, it appears that the concentration of the mercuric ion is not the only factor which determines the germicidal power. Paul and Krönig found that mercuric nitrate, although dissociated to a much greater extent than mercuric chloride, has a much weaker germicidal effect. According to Höber, this is due to the fact that of the two salts the chloride alone is soluble in the lipoid substances of which the living cell membrane consists; in virtue of this it is able to get

[1] Interesting evidence as to the specific action of the mercuric ion is supplied by Senter's study of the influence of various substances on the catalytic efficiency of hæmase (*Zeit. physikal. Chem.*, 1905, 51, 673). It was found that hydrocyanic acid and mercuric chloride, which are partly dissociated substances, paralyse the activity of hæmase much more powerfully than mercuric cyanide, which is practically undissociated.

at the protoplasm inside much more rapidly than the nitrate, and its power of penetration more than makes up for its deficiency of mercuric ions. Whether this be so or not, it is evident that the specific character of an ion, as regards germicidal action at least, is liable to be masked by the intervention of other factors. This is what happens in the case of acids regarded as disinfecting agents. Acids are alike in that when dissolved in water they all yield hydrogen ions to a greater or less extent, and it has been shown by Paul and Krönig that the germicidal effect of an acid is in the first place determined by its degree of dissociation, that in fact the hydrogen ion has a specific toxic action.[1] The weaker acids, however, are more toxic than we should expect if there were a complete parallelism between germicidal power and degree of dissociation; acetic acid, for instance, which from the figures recorded on p. 131, is seen to be feebly dissociated, is in regard to toxic power not far behind hydrochloric acid, which is highly dissociated. Here, according to Overton, it is the solubility of the undissociated molecules of the organic acids in the plasmatic membrane which accounts for their exceptional toxic power.

A case where it is pre-eminently the undissociated molecule of an acid, and not the hydrogen ion, which exerts a specific action is found in connection with artificial parthenogenesis.[2] It appears from Loeb's investigations that unfertilised eggs of *Strongylocentrotus purpuratus*, when placed for $1\frac{1}{2}$–2 minutes in a mixture of 50 cub. cm. sea-water + 3 cub. cm. $\frac{N}{10}$ butyric acid (or other monobasic fatty acid), and then replaced in normal sea-water, develop a typical fertilisation membrane. The

[1] Compare Senter, *loc. cit.*

[2] Loeb; see p. 73.

minimum concentration of monobasic fatty acid necessary for the production of the membrane diminishes as the number of carbon atoms in the molecule of the acid increases. Further, the strong mineral acids, hydrochloric, sulphuric, and nitric acids, are much less effective than the monobasic fatty acids; it was found that so far as inducing the formation of a membrane is concerned, $\frac{N}{1000}$ butyric acid is more effective than $\frac{N}{12}$ HCl. All the evidence, in fact, goes to show that it is the undissociated acid molecule which penetrates the egg and brings about the formation of the membrane.

Hydrogen and Hydroxyl Ions.—The hydrogen ion has been alluded to as possessing a specific toxic power, but all the characteristic properties of acid solutions are to be regarded as associated specially with this ion. Similarly the hydroxyl ion, present in the solutions of bases, confers on these solutions certain well-marked properties, which are to be regarded as characteristic of this ion.

The hydrogen and hydroxyl ions merit more detailed consideration, not only because acids and bases are such important groups of electrolytes, but also because these ions are exceptional in various ways. Reference to the table of ionic conductivities shows that these two ions are far and away more mobile than any others, either cations or anions. This fact, as the argument on p. 157 shows, involves the consequence that in regard to diffusive power acids and bases surpass all salts. Again, the hydrogen and hydroxyl ions are those which by their combination yield a molecule of water, and it has been suggested that this circumstance has something to do with their exceptionally high ionic velocities. The hydrogen ion, it is supposed, travelling under the in-

fluence of an electric force through an aqueous solution of an acid, collides with the anion side of a water molecule and displaces the hydrogen from the other side. This new hydrogen ion carries on the charge until it collides with a water molecule, when the process is repeated. In this way, it is supposed, the real distance to be traversed between the two electrodes is shortened, so that the mobility of the hydrogen ion appears to be greater than it really is.[1]

In regard also to their power of acting as catalytic agents the hydrogen and hydroxyl ions occupy an exceptional position. There are numerous reactions, for instance, which take place with appreciable rapidity only in the presence of acids, and it is found that the rate of the reaction is approximately proportional to the concentration of the hydrogen ions. The inversion of sucrose is a case in point, and the parallelism between the velocity of this change under the influence of various acids and the concentration of the hydrogen ions in each case is clearly shown by the following table.[2] The figures refer to equivalent quantities of the various acids, and hydrochloric acid is taken as the standard in connection both with the velocity and the hydrogen ion concentration. The actual method of measuring the velocity of inversion will be discussed later, but for the present the figures in Column I. may be accepted as representing the relative velocities of inversion under the influence of equivalent quantities of different acids. Instead of the hydrogen ion concentrations there are recorded in Column II. the relative conductivities of the acids in equivalent concentration. A strict measure of the hydrogen ion concentration would be given by

[1] Tijmstra, *Zeit. physikal. Chem.*, 1904, 49, 345; Danneel, *Zeit. für Elektrochem.*, 1905, 11, 249; Dempwolff, *Physikal. Zeit.*, 1904, 5, 637.

[2] Ostwald, *Journ. prakt. Chemie*, 1884, 30, 95.

a, the degree of dissociation in each case, but $a = \frac{\lambda}{\lambda_\infty}$, and as the value of λ_∞ does not vary very much from one acid to another, the conductivity of each solution may be taken as an approximate measure of the hydrogen ion concentration.

	I.	II.
HCl	100	100
$CHCl_2.COOH$	27	25
$CH_2Cl.COOH$	4·8	4·9
H.COOH	1·5	1·7
$CH_3.COOH$	0·40	0·42

The parallelism between the two sets of figures is unmistakable.

There are other reactions, such as the hydrolysis of esters, which are accelerated by acids, and the velocity of which is approximately proportional to the concentration of the hydrogen ion. The acceleration of these reactions appears therefore to be a specific property of the hydrogen ion, not of acids as such. If this view is accepted, then a sucrose solution, or a solution of methyl acetate, may be regarded as a reagent for hydrogen ions. The presence of these ions in any fluid may be detected and their amount estimated by studying the influence of this fluid on the inversion of sucrose or the hydrolysis of methyl acetate. The information given by such an investigation is quite different from that given by a titration of the fluid with a standard alkali solution; by this latter operation we ascertain merely the *total* acid present, both in the dissociated and undissociated conditions.

Various reactions are similarly available for the detection and estimation of the hydroxyl ion. It is well known that esters are readily saponified or hydrolysed by alkalis, and closer investigation of the problem has hown that the rate of saponification depends, not on

the total alkali concentration, but on that of the hydroxyl ions. The equation for the hydrolysis of ethyl acetate by sodium hydroxide in dilute aqueous solution is generally written

$$CH_3.COOC_2H_5 + NaOH = CH_3.COONa + C_2H_5OH,$$

but since sodium hydroxide and sodium acetate are both dissociated to a large extent under these conditions, the change would, from the point of view of the electrolytic dissociation theory, be more correctly represented by

$$CH_3.COOC_2H_5 + Na^{\cdot} + OH' = CH_3.COO' + Na^{\cdot} + C_2H_5OH.$$

Since $Na^{\cdot}$ occurs on both sides of the equation, it may be simplified to

$$CH_3.COOC_2H_5 + OH' = CH_3.COO' + C_2H_5OH.$$

In harmony with this form of the equation it is found, as already stated, that the velocity of saponification of ethyl acetate or other ester by a base depends on the concentration of the hydroxyl ion in the solution, and is practically independent of the positive radical of the base. By way of illustration the following figures may be quoted. The velocity of saponification of ethyl acetate by $\frac{N}{40}$ KOH at 24·7° is represented by the number 6·41; the corresponding figure for $\frac{N}{40}$ NH_4OH at the same temperature is found to be 0·16. Since the degrees of dissociation for $\frac{N}{40}$ KOH and $\frac{N}{40}$ NH_4OH are respectively 0·97 and 0·027, then on the assumption of an exact proportionality between velocity of saponification and hydroxyl ion concentration we should expect for the velocity of saponification by $\frac{N}{40}$ NH_4OH, the value

$\frac{6{\cdot}41 \times 0{\cdot}027}{0{\cdot}97} = 0{\cdot}18$. This figure is in good agreement with the value actually observed.

A reaction of a different kind which is accelerated by hydroxyl ions is the change of diacetonalcohol into acetone.[1] The velocity of this change is found to be proportional to the concentration of the hydroxyl ions, which act purely as catalytic agents, like hydrogen ions in the inversion of sucrose. The change diacetonalcohol →acetone furnishes, therefore, a means of detecting the presence and measuring the concentration of hydroxyl ions as distinct from undissociated bases. It should be noted, however, that in the case of weak bases, such as ammonia, there is not by any means exact proportionality between the velocity of change and the concentration of the hydroxyl ions.

The Dissociation of Water.—Reference has already been made to the interest which attaches to the hydrogen and hydroxyl ions on the ground that by their combination they yield water. So far, in fact, as aqueous solutions are concerned, these ions stand in a special relationship to the solvent. The question thus arises: Does water itself contain hydrogen and hydroxyl ions, and if so, what is the extent of the dissociation? It is evident that in any case the proportion of hydrogen and hydroxyl ions in water must be comparatively small, for, according to the view already adopted, an electric current is able to pass through an aqueous solution only in so far as there are ions present. Pure water is a very poor conductor. We may compare, for instance, the specific conductivity of good distilled water, which is about 7×10^{-6} at 18°, with that of normal sodium chloride solution, which is $7{\cdot}44 \times 10^{-2}$ at the same temperature. In other terms, distilled water enclosed in a centimetre cube, two opposite

[1] Koelichen, *Zeit. physikal. Chem.*, 1900, 33, 129.

faces of which act as electrodes, offers a resistance of about 140,000 ohms, while normal sodium chloride solution, under the same conditions, offers a resistance of 13·4 ohms. The conductivity of ordinary distilled water, moreover, arises chiefly from the impurities which it contains, notably carbon dioxide, and may be considerably reduced by simple methods. Kohlrausch has shown that when a current of air carefully freed from carbon dioxide is aspirated for some time through a sample of distilled water the conductivity of the latter is materially diminished, a result due to the removal of the greater part of the carbon dioxide. This gas may be got rid of also by redistilling the water and rejecting the first portion of the distillate, in which naturally the more volatile impurities of the water are concentrated. In such an operation great care must be taken that the mechanical carrying over of solid or liquid particles from the boiler is avoided by the introduction of a trap, and that the condensed water is brought into contact only with tin or the best kind of glass. An apparatus which claims to fulfil these conditions has been described by Hartley, Campbell, and Poole,[1] and it is instructive to note the values which they record for the conductivity of the distillates obtained at various stages of the distillation. The boiler used was capable of holding about 10 litres, and it was found that a considerable portion of this had to be distilled off before a fraction of very low conductivity was obtained. This is shown by the following figures:—

Time from Start of Boiling.	Quantity of Fraction Collected.	Specific Conductivity of Fraction.
Hours.	Litres.	$\kappa \times 10^6$.
0·5	0·5	>2
2·5	2·0	1·7
5·5	2·75	0·73
6·5	1·0	1·0

[1] *Journ. Chem. Soc*, 1908, 93, 428. Compare Bourdillon, *ibid.*, 1913, 103, 791.

The middle portion of the distillate is the purest, and is that which should be employed in the preparation of solutions for conductivity work (see p. 126). Nothing is to be gained by producing still purer water for ordinary investigations of conductivity, because even a sample for which $\kappa \times 10^6 = 0{\cdot}75 - 1{\cdot}0$ deteriorates during the contact with air which unavoidably occurs in the transference from the storage flask to the conductivity cell.

It is, however, an interesting question how far water can be freed from adventitious electrolytic impurities, and whether the residual conductivity then obtained is to be attributed to the dissociation of the water itself. Kohlrausch has pushed the purification of water to its utmost limit, and by distillation of a specially purified sample *in vacuo* has obtained water for which $\kappa \times 10^6 = 0{\cdot}04$ at 18°. If this figure is taken as representing the conductivity of absolutely pure water, then it is possible to calculate the concentration of the hydrogen and hydroxyl ions in this liquid. For this purpose we may regard pure water as a very dilute solution containing hydrogen and hydroxyl ions, and the equivalent conductivity must, according to Kohlrausch's law, be very nearly equal to $u_{\mathrm{H}} + v_{\mathrm{OH}} = 318 + 174 = 492$, for the ionic conductivities of the two ions at 18° are respectively 318 and 174. Now $\kappa \times \phi = \lambda$, where ϕ is the volume in cub. cm. which contains 1 gram-equivalent of each ion, and $\phi = \frac{\lambda}{\kappa} = \frac{492}{0{\cdot}04 \times 10^{-6}} = 12{\cdot}3 \times 10^9$ cub. cm. $= 12{\cdot}3 \times 10^6$ litres. That is to say, the quantity of water which contains 1 gram of hydrogen in the ionic form is over 12 million litres. The significance of this figure is open to criticism, in so far as Kohlrausch's investigations furnish of themselves no definite proof that the figure $0{\cdot}04 \times 10^{-6}$ is the conductivity of absolutely pure water. By three other independent methods, however, a value has been deduced

for the dissociation of water which is in good agreement with that calculated from the conductivity. There are therefore reasonable grounds for the view that the residual conductivity observed by Kohlrausch for his purest water is to be attributed to the dissociation of the water itself, and not to any impurities which it still contained.

Complex Ions.—In the earlier part of this chapter reference has been made to the additive character of the reactions of salt solutions—the fact on which the practice of the analytical chemist is based: the wet reactions so frequently employed are tests for the presence of various ions, not for salts as a whole. In this connection it is noteworthy that a metal which enters into the composition of a dissolved salt, although it generally forms the cation of the solution, does not do so invariably. Frequently it becomes part of a complex anion, and as each ion has its characteristic reactions, the tests which are employed to recognise the metal in the cationic condition gave no result in this case. A simple illustration is furnished by silver in potassium cyanide solution. If potassium cyanide is added to a solution of silver nitrate a precipitate of silver cyanide is obtained, which, however, dissolves up again when excess of potassium cyanide is added. The solution so prepared does not answer to the ordinary test for silver: sodium chloride may be added without producing any precipitate. The natural conclusion is that there can be no appreciable quantity of silver ions in the solution, and the question then arises: In what form is the silver present? The answer to this question was furnished long ago by Hittorf, who showed that when a solution of silver cyanide in potassium cyanide is electrolysed the silver migrates from the cathode to the anode, while of course in the case of an ordinary silver salt solution the silver travels as cation from anode

to cathode. In the cyanide solution, therefore, the silver must be part of the anion, and when the changes of concentration occurring at the electrodes during electrolysis are determined, it appears, as Hittorf showed, that the ions are $\overset{+}{K}$ and $\overline{Ag(CN)_2}$.

The transport of a metal from cathode to anode as part of a complex anion may be demonstrated directly in those cases where the anion in question possesses a characteristic colour. It is possible, for instance, to detect easily by an electrolytic experiment the difference between copper in a solution of copper sulphate and copper in Fehling's solution.[1] Two thistle funnels are sealed together so that the total length of the tube between the bulbs is about 30 cm. The tube is then bent as shown in Fig. 21. A normal solution of sodium chloride in 12

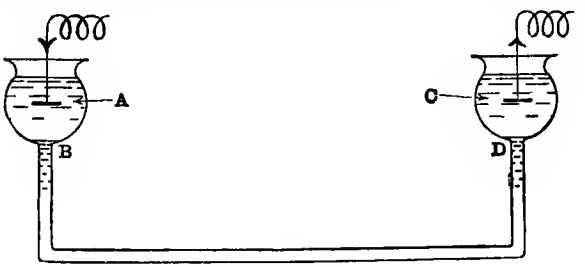

FIG. 21.

per cent. gelatin is run into the tube from B to D and allowed to set. One of the bulbs, A, is then filled with copper sulphate solution, the other, C, is charged with the deep blue neutral solution which is obtained when cupric tartrate is dissolved in caustic potash, excess of the alkali being avoided. Platinum electrodes are immersed in these two solutions, and are so connected with a source of current, that the electrode which dips in

[1] See Masson, *Journ. Chem. Soc.*, 1899, 75, 725.

the copper sulphate solution acts as anode. The E.M.F. applied to the electrodes ought to be about 30 volts, and the tube containing the jelly is kept in cold water during the experiment. After the current has passed for some time it is observed that the end of the jelly column next A is coloured pale blue; that is, the copper ions are migrating from anode to cathode. The other end of the jelly column, however, is also coloured blue, of a deeper shade, showing that complex ions containing copper are moving from cathode to anode.

A similar method has been employed by Donnan and Bassett[1] to show that the blue colour exhibited by cobalt chloride solutions under certain conditions is to be attributed to the presence of a complex anion containing cobalt.

Evidence of the formation of a complex salt is frequently found in an increase of solubility. As a general rule, the solubility of a salt is diminished in presence of another salt with a common ion; thus, for example, sodium chloride is less soluble in hydrochloric acid than in pure water at the same temperature. There are, however, numerous cases where an increase of solubility occurs on adding a salt with a common ion; thus silver cyanide is soluble in potassium cyanide, and mercuric chloride is more soluble in sodium chloride solution than in pure water. In both these cases complex salts are formed, and the metal becomes part of the anion, so that the concentrations of $Ag^{\cdot}$ and $Hg^{\cdot\cdot}$, even if not quite nil, are exceedingly small.

In view of this, it is only natural that, as shown by Paul and Krönig,[2] the germicidal power of a solution containing 1 mol. $AgNO_3 + 2$ mols. KCN in 4 litres is exceedingly small compared with that of a solution con-

[1] *Journ. Chem. Soc.*, 1902, 81, 939.

[2] *Zeit. physikal. Chem.*, 1896, 21, 425.

taining 1 mol. $AgNO_3$ in 4 litres. The silver ion, which is responsible for the toxic effect, is present in the first solution only to a very small extent. Similarly, the germicidal power of mercuric chloride is much reduced when sodium chloride is present in the solution; the addition of the latter salt involves the disappearance of mercuric ion as such, and its conversion into a complex anion of weak toxic power.

Another case of the formation of complex ions may be mentioned. It is well known that a solution of copper sulphate to which sucrose has been added fails to answer to the ordinary tests for copper; potassium hydroxide may be added to the solution without causing any precipitate. In harmony with this it has been found by Kahlenberg,[1] that in a solution containing sucrose, copper sulphate, and potassium hydroxide in the proportions represented by $C_{12}H_{22}O_{11} + CuSO_4 + 3KOH$ there are practically no copper ions. Further, Kahlenberg and True have shown[2] that while seedlings of *Lupinus albus* L. are killed in a solution containing a very minute quantity of copper ion, they are able to grow in a solution containing sucrose, copper sulphate, and potassium hydroxide in the afore-mentioned proportions, even when the amount of copper present is as much as $\frac{1}{400}$th of a gram-atom per litre. It makes, in fact, a great deal of difference whether the copper is present as $Cu^{\cdot\cdot}$ or as part of a complex ion.

[1] *Zeit. physikal. Chem.*, 1895, **17**, 612.
[2] *Bot. Gazette*, 1896, **22**, 81.

CHAPTER IX

COLLOIDAL SOLUTIONS

Crystalloids and Colloids.—In the preceding chapters dealing with the physical and biological characteristics of aqueous solutions reference has been made almost exclusively to solutions of such substances as sugar, salt, glycerine, acetic acid, and potassium nitrate. These compounds have been classed as electrolytes or non-electrolytes, according to the osmotic activity of their solutions, and their power to conduct the electric current. There is, however, a large class of substances which, on account of their special characteristics, must be distinguished both from electrolytes and non-electrolytes, as these terms are ordinarily understood. It was Graham who first made this distinction, and pointed out that substances which crystallised readily from water were characterised by high diffusive power, and by the ability to pass through animal or vegetable membranes; those substances, on the other hand, which cannot easily be obtained in the crystallised condition, amorphous substances in fact, are characterised by low diffusive power and by inability to pass through animal and vegetable membranes. The substances belonging to the first class, such as sucrose or sodium chloride, Graham termed *crystalloids;* those belonging to the second class, such as starch, gum, albumin, and caramel, he termed *colloids.* Solutions of these latter substances differ in many respects from those of crystalloids; they are of great interest and importance on both physical and

biological grounds, and they merit special consideration from the point of view adopted in this volume.

At the outset it ought to be explained that the term 'colloid' is now employed in a sense somewhat different from that in which Graham used it. It is generally interpreted at the present time as referring, not so much to a certain class of substances, but rather to a condition which a large number of chemical compounds may assume more or less readily.[1] A 'colloidal solution,' therefore, is not necessarily a solution of a colloid (in Graham's sense); it is to be interpreted as a solution the special characteristics of which are similar to those, say, of a gum, but the dissolved substance may be quite outside the class which Graham termed 'colloids'; it may be, for instance, ferric hydroxide, arsenious sulphide, or platinum. These latter substances, it is true, differ from gum, albumin, &c., in that they can be persuaded to form a colloidal solution only in an indirect way; mere contact with water, however prolonged, will not bring ferric hydroxide or arsenious sulphide into solution. The precipitation of these substances, therefore, from their colloidal solutions cannot be directly reversed, and they are accordingly sometimes termed 'irreversible' colloids, in contrast to gum, albumin, &c., which belong to the class of 'reversible' colloids, and are directly soluble in water. There are other points of contrast between a reversible and an irreversible colloid, which will appear later.

The substance which forms a colloidal solution is, as Graham showed, characterised by inability to pass through an animal or vegetable membrane, and on this fact is based the use of dialysis as a means of preparing a colloidal solution, free from dissolved crystalloids. A

[1] See Ostwald, *Grundriss der allgemeinen Chemie* (1909), p. 548.

piece of parchment or bladder is tied over one end of a wide glass cylinder, and into the receptacle so formed, a 'dialyser,' as it is called, the solution which is to be purified from crystalloids is poured. The lower end of the dialyser is then immersed in water, into which the crystalloids gradually diffuse through the membrane closing the dialyser. If the water is frequently renewed the colloidal solution inside is soon practically free from salts or other crystalloids, although it is very difficult to remove the last traces of these substances. Instead of a dialyser of the kind just described, a simple tube made of parchment paper may be employed. Charged with the colloidal solution, it is hung up by its ends and suspended in a vessel through which pure water is kept flowing. As an example of the use of dialysis, the preparation of a solution of silicic acid may be taken. When a solution of sodium silicate is poured into excess of hydrochloric acid, the silicic acid which is formed remains in solution along with sodium chloride and the extra hydrochloric acid: the mixture is subjected to dialysis, in the course of which the sodium chloride and hydrochloric acid diffuse out through the membrane of the dialyser, leaving behind a colloidal solution of silicic acid. Dialysis may be similarly employed in preparing a colloidal solution of ferric hydroxide, or in freeing a solution of egg albumin from admixed salts.

As Graham pointed out, there is a marked difference in the diffusive power of crystalloids and colloids. This appears from the following figures, which represent the relative times required for equal diffusion of two crystalloids and two colloids, sodium chloride being taken as the standard of comparison: sodium chloride, 1; sucrose, 3; egg albumin, 21; caramel, 42. The diffusion of enzymes has recently [1] been investigated, and it appears that these

[1] Herzog, *Zeit. Electrochem.*, 1907, 13, 533.

substances, like others which form colloidal solutions, are characterised by low diffusive power.

Osmotic Pressure of Colloidal Solutions.—In an earlier chapter it has been suggested that the phenomenon of diffusion in solution is closely connected with osmotic pressure. If there is such a connection, it is to be expected that the solution of a colloid, characterised as it is by a low rate of diffusion, will exert at the most only a small osmotic pressure. This conclusion is confirmed by experimental work, as will appear from the examples quoted below. In some cases indeed colloidal solutions have been found to exhibit no osmotic activity whatsoever; Reid,[1] for instance, working with carefully purified albumin solutions, came to the conclusion that these exert no osmotic pressure. In any investigation of the osmotic activity of a colloid, the question whether the solution is absolutely free from electrolytes is of the utmost importance; an electrolyte is highly active material from the osmotic point of view, and a small trace present in a colloidal solution may easily lead to erroneous conclusions. As has been hinted already, it is not easy to remove the last traces of electrolytes from a colloidal solution, and there can be no doubt that the older determinations of the osmotic pressure of colloids, such as those made by Pfeffer on gum arabic, gave too high values, owing to the presence of electrolytic material. Recent investigators who have determined the osmotic pressure of colloidal solutions have devoted much more attention to the problem of purification, and the values given by them may be regarded as representing more closely the real osmotic pressure of the colloid.

The osmotic pressures of colloidal solutions of ferric

[1] *Journ. Physiol.*, 1904, 31, 438.

hydroxide have been determined by Duclaux,[1] and the values obtained are recorded in the following table. A

Per Cent. $Fe_2(OH)_6$.	Pressure in cm. Water.
1·08	0·8
2·04	2·8
3·05	5·6
5·35	12·5
8·86	22·6

glance at the figures will show how small the pressures are even for the 5·35 per cent. and the 8·86 per cent. solutions: the pressure developed in the latter case is that which would be given by a solution containing about one-thirtieth of a gram of sucrose in 100 grams of water. The figures recorded in the table show that although the osmotic pressure increases with the concentration, there is not anything like proportionality between the two variables.

More attention has been devoted to the question of the osmotic activity of the serum proteins, egg albumin, and gelatin. In his experiments on the osmotic pressure exerted by serum proteins, Starling[2] employed an osmometer, the semi-permeable diaphragm of which consisted of peritoneal membrane soaked in gelatin and supported by silver wire gauze. The osmometer was charged with a solution of the serum proteins, the other side of the membrane being bathed by a salt solution which was approximately isotonic with the colloid solution in the osmometer. As the gelatin membrane was freely permeable to salts, the pressure observed in the osmometer was attributed to the colloids; the conclusion reached was that the osmotic pressure of blood serum containing 7–8 per cent. of proteins amounts to 25–30 mm. of mercury.

[1] *Compt. rend.*, 1905, 140, 1544.

[2] *Journ. Physiol.*, 1895, 19, 312; 1899, 24, 317.

Reid,[1] using a similar osmometer with a formalised gelatin membrane, found that when precipitates or crystals of protein are repeatedly washed with salt solutions, and the osmotic activity of samples of the material is investigated at intervals, the pressure observed for 1 per cent. protein concentration falls off steadily with continued washing; ultimately, as already indicated, solutions of protein are obtained which give no osmotic pressure. Hæmoglobin, however, was found to give a fairly definite pressure, amounting to 3·51–4·35 mm. of mercury for 1 per cent. concentration of hæmoglobin. The former observations would seem to indicate that the osmotic pressure frequently found for protein solutions is due, not to the proteins themselves but to something associated with them which may be gradually removed. It is, however, noteworthy, on the other hand, that the osmotic pressure developed by a colloidal solution is in many cases remarkably steady; this is difficult to explain if the pressure is attributed to crystalloids associated with the colloid, for the membranes employed in studying the osmotic activity of colloids are all readily permeable to crystalloids, so that these could produce at the most only a temporary effect.

As an example of the persistence of the osmotic pressure developed by a colloid, an observation made by Moore and Roaf[2] may be quoted. These investigators have studied in detail the behaviour of gelatin solutions in an osmometer closed with a parchment membrane. This osmometer consisted of two platinum capsules supported and held in opposition by brass chambers. The rim of each capsule was provided with a flange, and between the flanges there came, when the apparatus was put together, a thick platinum grid,

[1] *Loc. cit.*

[2] *Biochem. Journ.* 1906, **2**, 34.

supporting the membrane of parchment paper. Fig. 22 shows the osmometer fitted up and joined to the manometer. In one experiment the osmometer was charged with 10 per cent. gelatin, and a week later the osmotic pres-

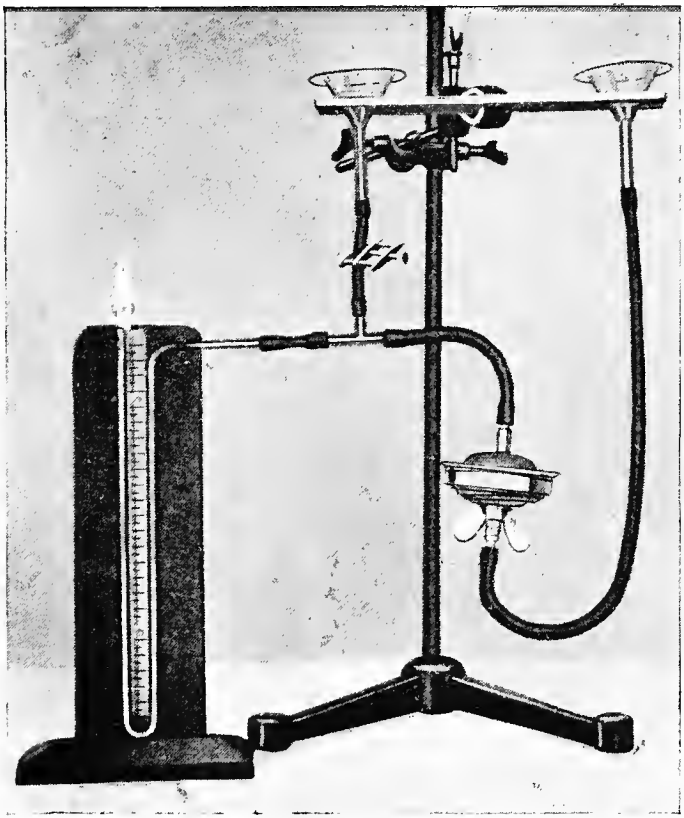

FIG. 22.

sure was found to be 74 mm. of mercury at 31° C.; the experiment was continued for two months, during which time the outside of the parchment membrane

was constantly bathed with water. At the end of that period the osmotic pressure was found to be 70 mm. of mercury at 26° C. Such a persistence of the pressure seems to show that it corresponds to a real equilibrium, and is not merely a passing phenomenon due to slowly diffusing crystalloids.

Other interesting observations made by Moore and Roaf relate to the influence of temperature on the osmotic pressure of a gelatin solution. The osmotic pressure increases with rising temperature, but the increase is more rapid than it would be if the osmotic pressure were strictly proportional to the absolute temperature. Further, if a gelatin solution, after being kept for a short time at 70–80°, is cooled, say, to 25°, the value then observed for the osmotic pressure is considerably higher than it was before the solution was heated. Only after the solution has been kept for some days at the lower temperature is there a return to the former value. The osmotic pressure exhibited by a gelatin solution is therefore to some extent dependent on its previous history. These observations seem to be most satisfactorily interpreted by supposing that the gelatin solution contains large molecular groups or aggregates, which tend to break up as the temperature rises, thus leading to an abnormally great increase of the osmotic pressure. When the solution is cooled the large molecular aggregates corresponding to the lower temperature are, it may be supposed, re-formed only slowly, and until this is complete the osmotic pressure is higher than the true equilibrium value.

This phenomenon of 'hysteresis' in connection with the osmotic activity of substances in colloidal solution indicates that the osmotic pressure in such a case is not completely defined by the two factors concentration and temperature. It is probable, as already suggested,

that the extent of aggregation of the colloid depends to some extent on its previous history; this being so, the number of units or separate aggregates in the solution, and therefore also the osmotic pressure, would not always be the same at a given temperature.

Striking evidence that the osmotic activity of a colloid depends on other factors than concentration and temperature is furnished by a recent investigation of the influence exerted by electrolytes on the osmotic pressure of colloidal solutions.[1] The colloids studied in this investigation were gelatin and egg albumin, and the osmometer used consisted of a flask-shaped vessel of collodion provided with a rubber stopper and vertical glass tube. A membrane of collodion is tough and only slightly extensible; it is practically impermeable to gelatin and egg albumin, but is readily permeable to all crystalloids. With this apparatus Lillie determined the osmotic pressure exerted by a colloid (1) when in a relatively pure condition, (2) when in the presence of crystalloids. The osmotic effect of the crystalloid in the latter case was eliminated by adding it also to the liquid outside the cell, so that the concentrations of the crystalloid on the two sides of the membrane were equal. It was then found that the osmotic activity of gelatin and egg albumin, while practically uninfluenced by sucrose and other non-electrolytes, is markedly affected by electrolytes. In presence of small quantities of either acid or alkali the osmotic pressure of a gelatin solution is notably increased, whilst that of an egg albumin solution is slightly lowered. Salts, on the other hand, bring about a lowering of osmotic activity for both colloids, the magnitude of the effect varying with the concentration and the nature of the salt. This may be illustrated by the following figures

[1] Lillie, *Amer. Journ. Physiol.*, 1907, 2270, 1.

for the osmotic pressure of 1·25 per cent. egg albumin and gelatin solutions:—

1·25 per Cent. Egg Albumin. Salt Present.	Osmotic Pressure in mm. Hg.	1·25 per Cent. Gelatin. Salt Present.	Osmotic Pressure in mm. Hg.
None	18·0	None	5·9
$\frac{N}{96}$ NaCl	6·8	$\frac{N}{96}$ NaCl	2·8
$\frac{N}{96}$ KNO_3	7·3	$\frac{N}{96}$ NaBr	3·1
$\frac{N}{12}$ NaCl	3·25	$\frac{N}{96}$ NaI	3·4
$\frac{N}{12}$ KNO_3	3·0	$\frac{N}{96}$ $NaNO_3$	3·0

It is apparent, then, that the osmotic activity of a colloid at a given concentration varies to a very marked extent with the nature and amount of crystalloid present. The lower pressures observed for egg albumin and gelatin in presence of salts can be attributed only to a reduction in the number of colloid units or aggregates in solution; so that the effect of salts, even in small quantities, is to increase the aggregation of the colloid. This is perhaps only natural, for it must be borne in mind that the addition of large quantities of salt to a protein solution leads frequently to the precipitation of the protein; the increased aggregation brought about by small quantities of salt may therefore be regarded as the first stage in a process which leads ultimately to precipitation.

Lillie's investigation indicates also that the phenomenon of hysteresis occurs in connection with the change in the aggregation of the protein which accompanies change in the amount of electrolyte present. The state of aggregation induced by a given electrolyte appears to persist to some extent even after the electrolyte has been removed, and it is at least possible that the very low values recorded by Reid[1] for the osmotic pressure of

[1] *Loc. cit.*

protein solutions were due to the treatment with concentrated salt solutions to which the protein was subjected.

The influence of electrolytes on the osmotic behaviour of colloids has been strikingly confirmed by a recent study of congo red.[1] This dye may be regarded as a colloid, inasmuch as it does not diffuse through parchment paper: in an electric field it moves towards the anode, and it is precipitated very readily by di- and trivalent cations. The osmotic activity of congo red, measured in the form of osmometer described by Moore and Roaf, is very nearly equal to that calculated on the supposition that it dissolves as single molecules, forming a true solution. Bayliss finds, however, that the theoretical osmotic pressure can be obtained only in the complete absence of extraneous electrolytes[2]; even the carbon dioxide present in ordinary distilled water brings about a notable fall in the osmotic pressure, owing to the aggregation of molecules to particles.

Molecular Weight of Colloids.—As has already been pointed out in an earlier part of this volume, the knowledge of the osmotic pressure of a solution enables us to calculate the molecular weight of the dissolved substance. Such a calculation is based on the assumption that osmotic pressure is proportional to concentration and absolute temperature. As shown, however, in the foregoing paragraphs, concentration and temperature are not the only factors which determine the osmotic pressure of a substance in colloidal solution.

It is therefore futile to deduce a value for the molecular weight of a colloid from the osmotic pressure of its solution: such a value could not be regarded as a characteristic figure for the colloid in question: it would

[1] Bayliss, *Proc. Roy. Soc.*, B, 1907, 81, 269.

[2] See also Biltz and Vegesack, *Zeit. physikal. Chem.*, 1909, 68, 357; 1910, 73, 481; Donnan and Harris, *Journ. Chem. Soc.*, 1911, 99, 1554.

have reference only to the particular conditions of the colloid at the time when the osmotic pressure was determined.

Values for the molecular weight of substances in colloidal solution may be deduced also from their effect on the vapour pressure, the boiling point, and the freezing point of water. Such values, however, must be accepted with reserve, on the grounds specified in the foregoing paragraph. There is, further, a special reason why little reliance can be placed on the indirect measurement of osmotic pressure in the case of a colloidal solution. If P is the osmotic pressure of an aqueous solution, and t is the extent to which the freezing point of the solution is lower than that of water, then, as shown on p. 110, $P = 12{\cdot}03t$ atmospheres. According to this formula, the freezing point of a solution which gives an osmotic pressure of 50 mm. of Hg—an easily measurable quantity—would be only about 0·005° below the freezing point of water. Such a small temperature difference might easily escape detection in ordinary work, and in any case the experimental error in its determination is relatively large. For the measurement, therefore, of the osmotic pressure of a colloidal solution the direct method is to be preferred. The values frequently quoted for the molecular weight of such colloids as dextrin, glycogen, and silicic acid—values based on the determination of the freezing point—have a very limited significance, partly on account of the deficiencies of the method employed, and partly on account of the variability in the aggregation of a colloid with the conditions.

Colloids in an Electric Field.—Nearly twenty years ago Linder and Picton[1] made the interesting observation that when two wires connected with the terminals of

[1] *Journ. Chem. Soc.*, 1892, 61, 148.

a battery were placed in a colloidal solution of arsenious sulphide the sulphide was attracted by the positive pole, and was gradually transported thither. Ferric hydroxide in colloidal solution was, on the other hand, attracted by the negative pole. It appears, therefore, that the particles of colloidal arsenious sulphide carry a negative electric charge, while those of colloidal ferric hydroxide carry a positive charge. The behaviour of colloids generally in an electric field has been extensively studied, and it is found that, as a rule, they carry a definite charge. In the following table various common colloids are classified according as they are electro-positive and move to the cathode, or electro-negative and move to the anode:—

Electro-positive.	Electro-negative.
Ferric hydroxide	Arsenious sulphide
Chromium hydroxide	Silicic acid
Methylene blue	Tannin
Bismarck brown	Caramel
Hæmoglobin	Starch
	Platinum
	Gold
	Indigo

The movement of colloids in an electric field may be demonstrated with the help of the apparatus described on p. 154. The bottom of the U tube is charged with a solution of caramel, for instance, the upper part of each limb being occupied by distilled water. When platinum wires connected with the terminals of a 200-volt circuit are put in the water of the two limbs, it is obvious after a long time that the column of caramel solution has fallen in one limb and risen in the other. If the wires are subsequently tested, it will be found that the one towards which the caramel advances is connected with the positive terminal of the circuit. A similar experiment might be made with any of the colloids mentioned above.

An apparatus of the same kind has been employed[1] to determine the actual rate at which colloidal metals migrate from cathode to anode under the action of an electric force. As shown by Bredig,[2] a colloidal solution of platinum, gold, or silver can be prepared by producing a small electric arc between wires of the metal in question when these are immersed in water. The passing of the discharge between the ends of the wires brings about what is known as the 'electrical pulverisation' of the metal; a coloured solution is obtained in each case, which can be filtered without change, and which can be purified by dialysis from any electrolytic impurities. The solution contains an appreciable quantity of metal, and is analogous in its properties to colloidal solutions of arsenious sulphide, ferric hydroxide, and many organic substances. If, now, the bend of the **U** tube of the apparatus referred to in the previous paragraph is filled with a colloidal solution of platinum, gold, or silver, and the upper parts of the two limbs contain water of the same specific conductivity as the colloidal solution, then when wires connected with a battery are put in the two water columns the potential gradient is uniform throughout the tube; the fall of potential per centimetre is known. Under the action of the electric force the colloidally dissolved metal moves towards the anode, and from the distance actually traversed in a given time the velocity of migration may be calculated for the potential gradient prevailing in the tube. In this way Burton has found that for a potential gradient of 1 volt per cm. the velocity of migration of colloidal platinum, gold, aud silver is about 0·0002 cm. per second, which is rather more than one-third of the rate at which the silver ion moves under similar conditions.

[1] Burton, *Phil. Mag.*, 1906, 11, 425.
[2] *Zeit. physikal. Chem.*, 1899, 31, 258.

One of the most significant facts bearing on the behaviour of colloids in an electric field is Hardy's observation[1] that protein in solution may be either electro-positive or electro-negative, according to circumstances. Hardy used the slightly opalescent fluid obtained when white of egg is mixed with 8–9 times its volume of water, filtered and boiled: this fluid is alkaline in reaction. When it is dialysed against distilled water, coagulation occurs, and the coagulum may be broken up and suspended in distilled water without solution taking place. If, however, a trace of acid is added, the flakes of the coagulum disappear, and an opalescent fluid with acid reaction is produced. If a current is passed through the original alkaline fluid, the protein moves from cathode to anode and a coagulum forms round the anode, while if a current is passed through the acid fluid described in the last sentence, the protein moves from anode to cathode. It appears, therefore, that in an alkaline medium the protein is electro-negative, but that in an acid medium it is electro-positive. It was found, further, that if the coagulum obtained by prolonged dialysis is thoroughly broken up and suspended in distilled water, no movement of the protein particles occurs in an electric field. If, however, the coagulum formed at the anode by passing a current through the original alkaline fluid is thoroughly broken up, the particles leave the anode and move towards the cathode; their electrical character has changed.

Such a reversal of the electric charge on a colloidal substance has been observed in other cases. Burton, for instance, working with colloidal solutions of silver and gold,[2] has found that the addition of small quantities of aluminium sulphate causes first a

[1] *Journ. Physiol.*, 1899, 24, 288.

[2] *Phil. Mag.*, 1906, 12, 472.

decrease in the charge on the colloidal particles, and ultimately a reversal.

Analogy between Colloidal Solutions and Suspensions.—The behaviour of substances in colloidal solution when exposed to the action of electrical forces is very similar to the behaviour of suspensions in the same circumstances. With the apparatus already described, it can be shown that when a current is passed through suspensions of quartz powder, gum mastic, or shellac, the suspended particles move towards the anode.

A great deal of other evidence is available in support of the view that there is a close relationship between colloidal solutions and ordinary suspensions. When we speak of a 'suspension' we may think of a fluid, in which distinguishable particles are floating about. There are, however, all grades of suspensions; many of them can be filtered without alteration, and in many cases no individual particles can be detected with the naked eye: the microscope at least is required to render the suspended particles visible. But if all the optical methods available for the recognition of the non-homogeneous character of a liquid are applied to colloidal solutions, evidence is obtained that these also contain distinct particles.

One of these methods consists in the application of the Tyndall test. It is well known that if a beam of light enters a darkened room in which dust particles are floating its path is rendered evident by the scattering of the light at the surface of the particles; each one of these appears as a bright moving speck. Similarly, when a powerful beam of light passes through a vessel containing a suspension, say, of fine silver chloride particles in water, its path is rendered evident by the scattering of the light which takes place at the surface

of the suspended particles; further, the light which is scattered is partly polarised. If, on the other hand, the beam is passed through a solution which is perfectly free from all suspended particles, no scattering of the light takes place, and the path of the beam cannot be detected; such a solution is described as 'optically empty.' Now the Tyndall phenomenon, that is, the appearance of opalescence which is observed when a powerful beam of light is passed through a fluid containing definite suspended particles, is exhibited also by the majority of colloidal solutions. Picton and Linder,[1] for instance, describe a colloidal solution of ferric hydroxide which, examined under a powerful microscope, appeared to be perfectly homogeneous, and which yet betrayed the track of a beam of light very distinctly, the light being completely polarised. A clear solution of hæmoglobin, similarly examined, gave a distinct soft luminous beam, the light of which was completely polarised. A positive result is obtained also with solutions of such substances as dextrin and gum arabic in water.

The extent of the analogy which thus appears to exist between suspensions and colloidal solutions has been thoroughly tested in recent years with the help of the ultramicroscope, as devised by Zsigmondy and Siedentopf.[2] The usefulness of this instrument depends on the Tyndall phenomenon, but whereas the power of any solution to exhibit the Tyndall phenomenon permits merely the conclusion that the solution contains distinct individual particles, the ultramicroscope makes it possible to detect the individual particles themselves, even where the most powerful microscope has failed to reveal any trace of heterogeneity. In the ultra-

[1] *Journ. Chem. Soc.*, 1892, 61, 148.

[2] See Zsigmondy's *Colloids and the Ultramicroscope*, p. 103.

microscope provision is made for focussing an intense beam of light in the liquid under examination, and in viewing the beam at right angles to its direction by a microscope. Any particles suspended in the liquid are then revealed in the field of the microscope as bright moving discs on a dim background.

The power of the ultramicroscope to detect discrete particles is considerably greater than that obtainable in ordinary microscopic methods. Particles of less than 140×10^{-6} mm. diameter are as a rule not visible under the microscope (compare the wave-length of red light, which is about 700×10^{-6} mm.), but with the ultramicroscope particles in a solution of colloidal gold as small as 4×10^{-6} mm. have been detected. One way in which the size of the particles revealed by the ultramicroscope may be estimated has recently been described by Burton,[1] and some figures may be quoted showing the method adopted by this investigator. A solution of colloidal silver containing 6·8 mg. of silver in 100 cub. cm. was diluted with water to 100 times its original volume. With the help of the ultramicroscope the number of particles in 0·1 cub. mm. of the diluted solution was found to be 300. Hence in 1 cub. cm. of the original solution there must have been 3×10^8 particles weighing $6{\cdot}8 \times 10^{-5}$ gram. If it is assumed that the particles are spherical and that their specific gravity is 10·5, then the mean radius is $1{\cdot}7 \times 10^{-5}$ cm. From experiments with colloidal platinum, gold, and silver, Burton found as the average diameter of the particles in these cases $2 \times 10^{-5} - 6 \times 10^{-5}$ cm. It is interesting to note that the smallest particle which can be detected in the ultramicroscope under the most favourable conditions (with bright sunshine) is about ten times as great as an

[1] *Phil. Mag.*, 1906, 11, 425. For a review of the methods used in determining the size of colloidal particles, see Henri, *Zeit. Chem. Ind. Kolloide*, 1913, 12, 246.

average chemical molecule, calculation having shown that the diameter of a chloroform molecule is about $0{\cdot}8 \times 10^{-6}$ mm., that of an ethyl alcohol molecule about $0{\cdot}4 \times 10^{-6}$, and that of a hydrogen molecule about $0{\cdot}1 \times 10^{-6}$.

The great majority of colloidal solutions, when examined in the ultramicroscope, are found to contain distinct particles. Cases are on record, however, in which the ultramicroscopic examination of colloidal solutions showed them to be optically empty. Zsigmondy, for instance, prepared a colloidal solution of gold which could not be shown to contain discrete particles. Such observations warn us that, while there is good ground for regarding suspensions and colloidal solutions as being closely allied, no hard-and-fast line of division can be drawn on the other hand between colloidal and crystalloidal solutions. For while, as already explained colloidal solutions can be prepared which are optically empty, there are solutions of crystalloids, sucrose, for instance, which cannot be obtained in this condition, however carefully they have been freed from suspended matter.[1] Strictly speaking, we must from the molecular standpoint regard all solutions as being ultimately non-homogeneous, and it is due to the inadequacy of our optical apparatus that we are unable to recognise separate particles in a dilute aqueous solution of ethyl alcohol or sodium chloride. One observation bearing directly on the question of the homogeneity of crystalloidal solutions has been recorded by van Calcar and Lobry de Bruyn,[2] who found that by rapidly centrifugalising sucrose solutions differences in concentration could be induced. Similar treatment of a saturated solution of sodium sulphate led to the crystallisation of some of the salt.

[1] Lobry de Bruyn and Wolff, *Rec. trav. chim.*, 1904, **23**, 155.

[2] *Rec. trav. chim.*, 1904, **23**, 218.

The inadvisability of attempting to put suspensions, colloidal solutions, and crystalloidal solutions in three absolutely distinct classes is emphasised by the fact that it is possible to prepare colloidal solutions of one and the same substance of all degrees of heterogeneity. Linder and Picton,[1] in their study of colloidal solutions of arsenious sulphide, showed that various 'grades' of solution could be obtained, according to the method of preparation.

They describe and distinguish the following:—

(α) Contained aggregates which were visible under the microscope.

(β) Was free from microscopically visible particles, but diffusion of the particles did not occur.

(γ) Contained invisible particles which diffused, but were kept back by filtration through a porous pot.

(δ) Contained invisible particles which diffused and were capable of passing through a porous pot. The solution, however, scattered and polarised a beam of light.

This example shows how far it is possible to vary the extent of aggregation of one and the same substance in colloidal solution. If it was a question of deciding whether a given arsenious sulphide solution was really a colloidal solution or only a suspension, the verdict would depend on the criterion of homogeneity employed: a solution which, according to one test, was a true colloidal solution would, according to another test, be merely a suspension. It is obvious, therefore, that, while there are no doubt broad distinctions to be drawn between suspensions, colloidal solutions, and crystalloidal solutions, the one class merges gradually into the other. The size of the individual particle

[1] *Loc. cit.*

present in a solution increases without any noticeable break from that, say, of an alcohol molecule in water, through that of the carbohydrates and proteins in aqueous solution, to cases where the individual particles are so large that we speak of them as 'suspended.'

Brownian Movement.—The invention of the ultramicroscope and the study of the phenomena exhibited by colloidal solutions have directed attention afresh to an observation which was made nearly a century ago by the botanist Robert Brown, and which has since then been the subject of repeated investigation. With the aid of the microscope Brown observed that fine particles suspended in water, such as gamboge or fat particles from milk, executed a vibratory movement about a mean position. This movement has been shown to be independent of any temporary forces due to slight differences of temperature or concentration; so long as the particles float in the liquid, the movement continues without ceasing.

The ultramicroscope has revealed the fact that a colloidal solution containing particles much smaller than Brown was able to observe is the scene of still greater activity. Zsigmondy, describing the movement of the gold particles in a gold hydrosol, compares them to a swarm of dancing gnats. The finer the particles, the more rapid is their movement; with increase in size the movement becomes slower, and it is ultimately imperceptible when the diameter of the particles is about 0·004 mm. Zsigmondy considers that in contradistinction to the typical Brownian movement about a mean position, the motion of the smallest gold particles in a gold hydrosol is continuous; an individual particle, after moving about in a zigzag fashion, may suddenly rush across the field like a living thing. The mean

free path is therefore considerably greater in the case of the smallest gold particles than it is in the ordinary Brownian movement, but there is no doubt that the phenomenon is essentially the same in the two cases.

The problem has recently been attacked by Svedberg,[1] who has prepared a number of colloidal solutions of platinum in various media and shown that the amplitude of vibration of the particles is nearly inversely proportional to the viscosity of the medium. The mean velocity, however, of a platinum particle of given size is practically constant; for a particle weighing about $2{\cdot}5 \times 10^{-15}$ gram the mean velocity is estimated to be 3×10^{-2} cm. per second at the ordinary temperature. Comparison of these figures with the corresponding ones given by Ramsay[2] for larger particles leads to a calculation of the velocity with which a platinum molecule would move. It is noteworthy that the value so calculated is in agreement with the value based on the assumptions of the kinetic theory. It is therefore probable that the Brownian movement of suspended or colloidal particles is an expression of the molecular movement which is attributed to matter generally. This view has been strengthened by still more recent work, both theoretical and experimental.[3] Indeed Ostwald[4] has expressed the opinion that the agreement between the observed and calculated values for the rate of movement of a suspended particle is so close as to amount to an experimental proof of the kinetic nature of heat.

Filtration of Colloidal Solutions.—Filtration is the

[1] *Zeit. Elektrochem.*, 1906, 12, 853.

[2] *Chem. News*, 1892, 65, 90.

[3] See Svedberg, *Zeit. Elektrochem.*, 1906, 12, 909; Perrin, *Compt. rend.*, 1908, 146, 967; 147, 475, 530; also Perrin's *Brownian Movement and Molecular Reality* (1910).

[4] *Grundriss der allgemeinen Chemie* (1909), p. 543.

time-honoured method of freeing a liquid from suspended particles, and the remarkable similarity between suspensions and colloidal solutions leads us naturally to inquire how far this method is efficient when applied to colloidal solutions. The inquiry really reduces itself to the question whether we can procure filters with sufficiently small pores. The analytical chemist knows that the possibility of filtering a very finely divided precipitate depends on the texture of the filter paper. In the case of colloidal solutions, which pass unchanged through the finest filter paper, the possibility of a mechanical separation of the colloid depends on the discovery of a filter with exceedingly small pores—of a diameter $1 \times 10^{-6} - 40 \times 10^{-6}$ mm. One such, as suggested by Martin, is obtained by impregnating the pores of a Pasteur-Chamberland candle with gelatin. A filter so prepared is highly permeable to crystalloids such as sodium chloride and butyric acid, but is very slightly permeable to colloids such as ferric hydroxide and soluble starch,[1] so that if a colloidal solution of ferric hydroxide is filtered under 100 atmospheres pressure in such an apparatus the filtrate consists of practically pure water. The permeability of such a filter to certain colloids increases as the concentration of the impregnating gelatin solution diminishes. This fact has been utilised in the attempts which have recently been made to differentiate between various colloidal solutions by means of graded filters. These, according to Bechhold's suggestion,[2] may be prepared (1) by impregnating filter paper with a solution of collodion in glacial acetic acid and then dipping in water, or (2) by soaking filter paper in gelatin solution and then hardening with formaldehyde. The size of

[1] See Craw, *Proc. Roy. Soc.*, B, 1906, 77, 311.

[2] *Zeit. physikal. Chem.*, 1907, 60, 257.

the pores in such gelatinised filters diminishes as the concentration of collodion or gelatin used in their preparation increases. A series of graded filters is thus obtainable which may be used to sort out a number of colloidal solutions according to the size of particles they contain. It is true that the pores in a filter are not all of equal diameter and that the particles in a colloidal solution vary in size, but still it is possible to discover for a given colloidal solution which one of the series of filters is just able to prevent the passage of the colloid. Such experiments obviously lead to a classification of colloidal solutions according to the size of the particles they contain, and the following table given by Bechhold is based on work of this kind:[1]—

Suspensions	Serum Albumin
Prussian Blue	Diphtheria Toxin
Colloidal Platinum	Protalbumose
Colloidal Ferric Hydroxide	Colloidal Silicic Acid
Casein (in Milk)	Deuteroalbumose
Colloidal Arsenious Sulphide	Litmus
Colloidal Gold (40×10^{-6} mm.)	Dextrin
1 per cent. Gelatin	*Crystalloids*
1 per cent. Hæmoglobin	

Although the value of this table is qualified by the fact that the size of the particles in the colloidal solution of a given substance varies with the mode of preparation, yet the order given is in general agreement with theoretical considerations and with the results of ultramicroscopic investigation.

[1] The pressures under which filtration took place in Bechhold's experiments were between 0·2 and 5 atmospheres.

CHAPTER X

THE SEPARATION OF COLLOIDS FROM THEIR SOLUTIONS

Suspension and Emulsion Colloids.—In the foregoing chapter a good deal of evidence has been brought forward showing that the region of colloidal solution adjoins that of true solution on the one side and that of suspensions on the other. When now we consider the influences which bring about a separation of the colloid from its solution, it is found that the substances which form colloidal solutions may be divided into two classes. In relation to precipitating or coagulating agents the one class resembles suspensions, while the other behaves more like crystalloidal substances.

The two classes are those which have already been referred to (p. 178) as irreversible and reversible colloids; they may be distinguished also as 'suspension colloids' and 'emulsion colloids,' or as 'suspensoids' and 'emulsoids.'[1] A colloid belonging to the suspensoid class gives with water a mixture which is non-viscous and non-gelatinising, but is coagulated on the addition of small quantities of electrolytes. A colloid belonging to the emulsoid class gives with water a mixture which is viscous, gelatinises, and is not readily coagulated by salts.

The Coagulation of Suspensoids.—One of the most characteristic features of a colloidal solution of arsenious

[1] von Weimarn, *Zeit. Chem. Ind. Kolloide*, 1908, 3, 26.

sulphide or ferric hydroxide is the ease with which these colloids are precipitated on the addition of electrolytes. A similar sensitiveness to small quantities of salts is exhibited by suspensions. Bodländer has shown that the sedimentation of kaolin suspensions is accelerated by the addition of electrolytes, and Hardy has found[1] that a suspension of gum mastic in water, prepared by adding a dilute alcoholic solution of the gum to distilled water, is precipitated immediately by very small quantities of magnesium sulphate or barium chloride. On the other hand, the stability of a suspension or the solution of a suspensoid is practically unaffected by the addition of a non-electrolyte.

In making a comparative study of the influence of various electrolytes in causing precipitation of suspensoids, it is necessary to follow a strictly uniform procedure. Experience has shown, firstly, that an amount of salt which is not capable of causing immediate coagulation is nevertheless effective after a certain interval, and secondly, that the total quantity of electrolyte required to bring about complete coagulation of the suspensoid varies according as the electrolyte is added all at once or in several portions successively. We have here an indication of the part which the time factor plays in the behaviour of colloidal solutions (compare p. 184). In order to avoid complications arising from these causes Freundlich has suggested the following procedure:[2]—2 cub. cm. of the electrolytic solution are added to 20 cub. cm. of the suspensoid solution, the latter being well shaken during the addition; the mixture is then allowed to stand for two hours, after which time a few cubic centimetres are filtered off, and the filtrate is examined, chemically or colorimetrically, for the suspensoid.

[1] *Zeit. physikal. Chem.*, 1900, 33, 385.
[2] *Ibid.*, 1903, 44, 131.

The following table records some of the results obtained by Freundlich in his investigation of the influence of electrolytes in precipitating a colloidal solution of arsenious sulphide. The tests were carried out as described in the previous paragraph, and the numbers given in the table represent the minimum concentration for each electrolyte which brought about coagulation in two hours; the figure given in each case is the concentration of the electrolyte after mixing with the arsenious sulphide solution.

Electrolyte.	Millimols. per Litre.
NaCl	71·2
KNO_3	69·8
$\frac{1}{2}K_2SO_4$	91·5
NH_4Cl	59·1
HCl	42·9
$MgCl_2$	1·00
$MgSO_4$	1·13
$Ca(NO_3)_2$	0·95
$BaCl_2$	0·96
$ZnSO_4$	1·13
$AlCl_3$	0·13
$Al(NO_3)_3$	0·14
$\frac{1}{2}Ce_2(SO_4)_3$	0·13

Inspection of this table shows, firstly, that exceedingly small quantities of electrolytes suffice to cause the coagulation of arsenious sulphide solutions; secondly, and more particularly, that the coagulating power of an electrolyte in relation to arsenious sulphide is mainly determined by the valency of the cation. The higher the valency of the cation, the smaller is the quantity of electrolyte required to bring about coagulation.

Equally striking is the comparative influence of salts in bringing about the coagulation of colloidal ferric hydroxide. This is shown by the following table,

the figures in which have the same significance as those quoted in the previous table. It is evident that in relation to colloidal ferric hydroxide the coagulating power of a salt is mainly determined by the valency of the negative ion; the valency of the positive ion is here relatively unimportant.

Electrolyte.	Millimols. per Litre.
$NaCl$	9·25
$\frac{1}{2}BaCl_2$	9·64
KNO_3	11·9
$\frac{1}{2}Ba(NO_3)_2$	14·0
K_2SO_4	0·20
$MgSO_4$	0·22
$K_2Cr_2O_7$	0·19

The contrast in this respect between colloidal arsenious sulphide and colloidal ferric hydroxide becomes still more interesting when it is borne in mind that the colloidal particles of arsenious sulphide are negatively charged, while those of ferric hydroxide are positively charged. The full significance of this was first appreciated by Hardy,[1] who formulated the rule that the ion of an electrolyte which determines the coagulation of a colloidal solution is the one which has a charge opposite in sign to that on the colloidal particles. The validity of this rule is strikingly confirmed by Hardy's experiments on the coagulation of the protein solution described on p. 191. It will be remembered that in a faintly alkaline medium this protein is electro-negative, while in a faintly acid medium it is electro-positive. It is accordingly found that in presence of a trace of alkali aluminium sulphate is much more effective than sodium sulphate in bringing about the coagulation of the protein, while magnesium sulphate occupies an in-

[1] *Zeit. physikal. Chem.*, 1900, 33, 385.

termediate position; when, however, the protein solution contains a trace of acetic acid, the three sulphates are about equally effective in causing coagulation. Similarly it is found that while barium chloride is more effective than sodium sulphate in coagulating the electro-negative protein, the positions of the salts are reversed in relation to the electro-positive protein.

All these results, taken in conjunction with the fact that non-electrolytes are ineffective, show that the coagulation of suspensoids is essentially a process in which ions are primarily involved. It is therefore not surprising to find that when various electrolytes all yielding the same cation are used to coagulate arsenious sulphide, the effectiveness increases with the degree of dissociation; the smaller the extent to which the electrolyte is dissociated, the greater relatively is the quantity of it required to bring about complete coagulation. This is shown in a general way by the following table;[1] the figures in the second column give the concentration necessary in each case to cause coagulation of a colloidal arsenious sulphide solution :—

Acid.	Gram-Equivalents per Litre.	Spec. Conductivity at 18° (comparative).
HCl	0·0038	14·5
HNO_3	0·0038	14·3
H_2SO_4	0·0043	13·2
$H_2C_2O_4$	0·009	14·4
H_3PO_4	0·015	13·9
$CH_3.COOH$	0·70	12·6

It is seen that although very different quantities of the acids must be taken to bring about coagulation, yet each of the coagulating solutions has approximately the same conductivity, that is, approximately the same number of ions.

If the coagulation of a suspensoid is the result of a

[1] Hardy, *loc. cit.*

neutralisation of electric charges, one on the colloid particles and one on the coagulating ion, then the precipitated colloid, the 'coagulum,' or 'hydrogel' as it may be called, ought to contain either the acidic or basic part of the added electrolyte. This is actually the case; Linder and Picton[1] found that when a colloidal solution of arsenious sulphide is precipitated by adding barium chloride, the coagulum contains barium. This barium cannot be removed from the precipitate by continued washing with water, but when the precipitate is treated for some time with a solution of another salt, the barium is replaced by the metallic part of this salt. Similar observations have been made by Whitney and Ober,[2] who show that when colloidal arsenious sulphide is precipitated by barium chloride, the quantity of barium carried down in the coagulum is independent of the concentration of the solution, but is proportional to the amount of sulphide precipitated. The composition of the coagulum obtained by Whitney and Ober is represented by $90As_2S_3 + 1Ba$, and in proportion as the coagulum retains barium the filtrate becomes acid. Further, when four equal quantities of colloidal arsenious sulphide are precipitated by barium, strontium, calcium, and potassium chloride respectively, the coagula retain equivalent quantities of the four metals. The retention of the precipitating metal by the coagulum appears to be a case of adsorption,[3] which will be discussed later.

Some interesting observations are on record dealing with the coagulation of colloidal arsenious sulphide by mixed electrolytes. The coagulating effect of the mixed chlorides of two uni-valent metals is simply the sum of the two separate effects; the effect, however, of a

[1] *Journ. Chem. Soc.*, 1895, 67, 63. [2] *Zeit. physikal. Chem.*, 1902, 39, 63.
[3] Freundlich, *Zeit. physikal. Chem.*, 1910, 73, 385.

mixture containing the chloride of a uni-valent metal and the chloride of a di-valent metal, is less than that calculated on the additive basis. There appears, therefore, to be a certain antagonism in this respect between uni-valent and di-valent cations, and it is worth noting that a similar antagonism has been observed in some physiological experiments made by J. Loeb.[1] This investigator found that freshly fertilised eggs of *Fundulus heteroclitus* when transferred from sea-water to an isotonic solution of pure sodium chloride all die without developing. If, however, there is first added to the pure sodium chloride a small quantity of the chloride of almost any di-valent metal, the resulting mixture is a more or less suitable medium for the development of fertilised Fundulus eggs. The toxic effect of pure sodium chloride is thus inhibited by salts with di-valent cation.

In the previous chapter it was suggested (see p. 186) that the increased aggregation of a colloid brought about by small quantities of a salt might be regarded as the first stage in a process which ultimately leads to precipitation. Now the precipitation of a positive colloid, as we have just seen, is determined chiefly by the negative ion of the added electrolyte, and the precipitation of a negative colloid by the positive ion of the added electrolyte. It might therefore be expected that the addition of an alkali (*i.e.* of OH' ions) to the solution of a positive suspensoid in quantity insufficient to produce coagulation would lead to an increase in the size of the suspensoid particles, and that a similar result would follow the addition of an acid (*i.e.* of $H^{\cdot}$ ions) to a negative suspensoid in quantity insufficient to produce coagulation. This has been verified by Mayer, Schaeffer, and Terroine,[2] who used the ultramicroscope to study the changes of

[1] *Amer. Journ. Physiol.*, 1902, 6, 411. Compare Osterhout, *Science*, 1911, 34, 187; 1912, 35, 112.

[2] *Compt. rend.*, 1907, 145, 918.

size exhibited by the colloid particles. They found further that the addition of $H^{\cdot}$ ions in small quantity to a positive colloid led to a *decrease* in the size of the particles, as did also the addition of OH' ions to a negative colloid. The colour changes exhibited by gold and silver hydrosols on the addition of minute quantities of electrolytes are similarly to be referred to alterations in the aggregation of the colloid particles.

Reciprocal Coagulation of Suspensoids.—The study of the influence of electrolytes on suspensoids has shown clearly that in the process of coagulation the charge on the colloid is neutralised by that on one of the ions of the added electrolyte. If this view of the coagulation process is correct, then we may fairly expect that if we neutralise the charge on the colloid in any other way, a similar result will follow. In the previous chapter evidence has been recorded showing that some colloidally dissolved substances carry a negative charge, while others carry a positive charge. It may therefore be reasonably expected that if the solution of a positive colloid is added to the solution of a negative colloid, (1) coagulation will result, and (2) the coagulum will contain both colloids, for, as already stated, the coagulum obtained when barium chloride is added to arsenious sulphide solution contains both arsenious sulphide and barium. The experiments carried out by Biltz[1] have verified both predictions. This investigator showed, first, that no coagulation occurs when hydrosols of the same electrical sign are mixed. When, however, a solution of a positively charged colloid is added to that of a negatively charged colloid precipitation occurs in all cases, unless the quantity of the added colloid is relatively either very small or very great. For a certain proportion

[1] *Ber. deut. chem. Ges.*, 1904, 37, 1095.

of the colloids precipitation of both is complete; as the quantities deviate from this optimal ratio, precipitation is increasingly incomplete. It is possible, for instance, to bring about the complete precipitation of arsenious sulphide from its solution by the addition of a suitable quantity of ferric hydroxide hydrosol; similarly, aniline blue, which forms a negative hydrosol, is precipitated by magdala red, which forms a positive hydrosol.

The precipitation of egg albumin by solutions of various complex acids—*e.g.* molybdic, tungstic, and tannic acids—furnishes an example of the mutual coagulation of two colloids.[1] Metaphosphoric acid, too, forms a pseudo-solution or hydrosol which precipitates albumin, while the crystalloidal orthophosphoric acid has no such effect.

An interesting case in which the reciprocal coagulation of two colloids has been employed for a practical purpose is furnished by a recent investigation of Michaelis and Rona.[2] They show that mastic suspension and a faintly acid solution of protein precipitate each other completely when they are mixed in a certain proportion; if they are mixed in any other proportion, the precipitation is incomplete. This observation is taken as the basis of a method for the removal of the last traces of proteins from blood serum. The bulk of the protein in the serum is precipitated with alcohol, and the filtrate, containing not more than 1 per cent. of protein and a trace of acetic acid, is treated with excess of mastic suspension. This of itself does not bring about complete precipitation of the protein, but if the excess of mastic is coagulated by the addition of a small quantity of an electrolyte, it carries down all the remaining protein with it. The filtered liquid is then free from both mastic and protein.

[1] See Mylius, *Ber. deut. chem. Ges.*, 1903, 36, 775; Biltz, *loc. cit.*

[2] *Biochem. Zeit.*, 1907, 2, 219; 5, 365.

The Precipitation of Emulsoids.—Emulsoids differ notably from suspensoids in their slight sensitiveness to the presence of neutral alkali salts. Comparatively small quantities of these are able to produce coagulation of arsenious sulphide or ferric hydroxide, while the quantities required to cause precipitation of, say, serum albumin from its solution are very great. The precipitation of an emulsoid by a neutral alkali salt is reversible, while the corresponding precipitation of a suspensoid is irreversible. Further, the factors governing the precipitation are quite different in the two cases. All the evidence goes to show that the precipitation of a suspensoid by an electrolyte is essentially electrical in character; the precipitation of a reversible colloid by a neutral alkali salt, on the other hand, has much in common with the phenomenon of 'salting out,' familiar especially to the organic chemist. Electrical influences have nothing to do with the precipitation of a reversible colloid, for as Pauli has shown,[1] protein from serum can be so purified by dialysis that even in a steep potential gradient it exhibits no tendency to migrate either towards the anode or towards the cathode, yet in this neutral condition the protein can be precipitated by alkali salts; hence it is clear that the factors which determine precipitation in this case are not electrical.

The grounds for regarding the precipitation of emulsoids by neutral alkali salts as allied to the phenomenon of 'salting out' rather than to the coagulation of suspensoids are to be found in the relative effects of these salts. The results of many investigations of the influence of salts on the solubility of hydrogen, carbon dioxide, ethyl acetate, and other sparingly soluble substances (compare p. 26), have shown that the effect of any particular salt is the sum of the effects of the ions;

[1] *Beitr. chem. Physiol. Path.*, 1906, 7, 531.

a diminution in the solubility of any of these substances is not due to the cation in one case, to the anion in another, as in the coagulation of suspensoids; each ion is responsible for part of the effect. By comparing the effects of a number of potassium salts in lowering the solubility of, say, hydrogen in water, it is possible to arrange the anions according to the magnitude of their influence; similarly, by comparing the effects of the chlorides of the alkali metals on the solubility of hydrogen, it is possible to arrange the cations according to the magnitude of their influence. The order of the anions determined in this way, beginning with the one which is most effective in lowering the solubility of hydrogen, &c., is SO_4'', Cl', Br', I', NO_3'; the corresponding order for the cations is $Na^{\cdot}$, $K^{\cdot}$, $NH_4^{\cdot}$. Hofmeister and Pauli[1] have studied in a similar fashion the influence of various alkali salts in precipitating proteins, and they find that the effect of a given alkali salt is an additive function of the two ions. When the ions are arranged according to the magnitude of their effects, the following series are obtained, the first member of each series being the most effective in causing precipitation: SO_4'', HPO_4'', $CH_3.COO'$, Cl', NO_3', Br', I', CNS'; $Li^{\cdot}$, $Na^{\cdot}$, $K^{\cdot}$, $NH_4^{\cdot}$. Comparison of these with the previous series shows that the order is very nearly the same in the two cases. The precipitation of reversible colloids by neutral alkali salts appears therefore to be closely allied to the process of 'salting out,' and, like the latter, is probably connected with the hydration of the salts. This result emphasises the fact that solutions of reversible colloids approximate more to true solutions that do solutions of suspensoids.

When a neutral solution of protein is made slightly acid or slightly alkaline, its properties undergo a marked

[1] See Pauli, *Beitr. chem. Physiol. Path.*, 1902, 3, 225. Compare Robertson, *J. Biol. Chem.*, 1911, 9, 303.

modification. In a potential gradient the protein now migrates towards the anode or cathode according as the medium is alkaline or acid, and in respect also to the precipitating power of salts its behaviour now resembles that of an ordinary suspensoid.[1] The properties of a protein solution containing a trace of alkali or acid have accordingly been discussed in an earlier paragraph of this chapter, where it was shown that in these circumstances it is the valency of the cation or anion which is the predominating factor in determining the precipitation. The modification in the character of protein which results from the addition of small quantities of acid or alkali is demonstrated also by its behaviour towards salts of the heavy metals. These are unable to precipitate carefully dialysed neutral protein, but as soon as the protein has acquired an electro-negative character, it is readily precipitated by small quantities of these salts; the precipitation, further, is irreversible in character, and therefore quite different from the precipitation of neutral protein by alkali salts.

The readiness of protein to change its character with the acid or alkaline reaction of the medium becomes intelligible on the basis of Hofmeister's theory, that the proteins are produced by the condensation of several amino acids, and that the protein molecule is characterised by the presence of at least one amino group and one carboxyl group. On this view protein is an 'amphoteric' electrolyte, that is, an electrolyte which may act either as an acid or as a base—which may split off either hydrogen or hydroxyl ions. According to circumstances, therefore, the protein molecule may assume either an acid or a basic function: it forms salts both with bases and with acids.

[1] Hardy, *Zeit. physikal. Chem.*, 1900, 33, 385; also Pauli, *Beitr. chem Physiol. Path.*, 1906, 7, 531.

Protective Action of Reversible Colloids.—When a reversible colloid is added to the solution of a suspensoid, the precipitation of the latter by electrolytes is more or less inhibited. This is not generally due to an increase in the viscosity of the medium, and consequently increased resistance to sedimentation, for the protective effect is produced by very small quantities of the reversible colloid, insufficient to cause any appreciable change of viscosity. As an illustration of this phenomenon we may take the influence of various reversible colloids on the stability of a gum mastic suspension. Bechhold has shown[1] that while a mixture of 1 cub. cm. mastic suspension + 1 cub. cm. 0·1N $MgSO_4$ made up to 3 cub. cm. with water is completely coagulated in 15 minutes, no coagulation occurs within 24 hours if 2 drops of a 1 per cent. gelatin solution are added before making up to 3 cub. cm.; the gelatin 'protects' the mastic. The coagulation of mastic suspension is similarly inhibited by ox blood serum and gum arabic.

An extreme case of this protective action is furnished by the colloidal silver halides described by Paal and Voss.[2] These are obtained by adding sodium halide to silver hydroxide in presence of sodium protalbate or lysalbate, salts which are prepared by the action of sodium hydroxide on egg albumin. The colloidal solutions of silver halide so obtained are opalescent, and yield a slightly coloured solid, containing as much as 90 per cent. of silver halide, and yet dissolving readily in cold water. It is probable that in such cases the emulsoid forms a thin envelope round each suspensoid particle, and so prevents the aggregation and consequent flocculation of the particles.

Reversible colloids differ appreciably in their power to

[1] *Zeit. physikal. Chem.*, 1904, 48, 408.
[2] *Ber. deut. chem. Ges.*, 1904, 37, 3862.

protect suspensoids from coagulation by electrolytes, and an attempt has been made by Zsigmondy [1] to differentiate various protein substances on this basis. A red solution of colloidal gold turns blue on the addition of sodium chloride and other salts owing to increase in size of the colloid particles, but this change of colour may be prevented by the presence of proteins. A more or less definite amount of each protein is required to secure this result, and the proteins may be classified correspondingly. For this purpose Zsigmondy used the 'gold number,' which is defined as the weight in milligrams of the reversible colloid which is just insufficient to prevent the change from red to blue in 10 cub. cm. of colloidal gold solution after the addition of 1 cub. cm. of 10 per cent. sodium chloride solution. How far the 'gold number' varies from one case to another will be seen from the following table:—

	Gold Number.
Gelatin	0·005–0·01
Caseinogen	0·01
Globulin	0·02–0·05
Egg albumin amorph.	0·03–0·06
Egg albumin cryst.	2–8
Fresh egg-white	0·08–0·15

It is noteworthy that albumoses are altogether unable to exert a protective action on the red solution of colloidal gold.

Colloids in Biology.—In view of the enormously important part played by colloids in the living cell itself and in all physiological fluids, it is obvious that a knowledge of the peculiar characteristics of these substances is a necessary preliminary to any effort to interpret vital phenomena. In recent years our knowledge of the pro-

[1] *Zeit. analyt. Chem.*, 1901, 40, 697; see also Schulz and Zsigmondy, *Beitr. chem. Physiol. Path.*, 1902, 3, 137.

perties of colloids has been growing rapidly, and numerous and noteworthy attempts have been made to use this new knowledge in attacking biological problems of various kinds. In this and the foregoing chapters, in which a brief account of the outstanding characteristics of colloids has been given, reference has been made incidentally to various cases in which the behaviour of colloids seems to have a direct bearing on certain biological phenomena. There are, however, numerous other problems in which colloids are essentially involved, and on which much new light has been thrown by the colloid investigations of the past ten or fifteen years.

In this period much work has been done with the object of elucidating the nature and mode of action of enzymes, toxins, and antitoxins, and as these are all colloids, it is only natural that attempts have been made to correlate their behaviour with that of less complex bodies of the same class. As a first example of such correlation we may take what is known as the Danysz phenomenon. The toxicity of a mixture of diphtheria toxin and antitoxin depends on the way in which the two are mixed. If the amount of toxin added is such that the mixture is non-toxic, then in a second experiment, in which the same amounts of antitoxin and toxin are taken, in which, however, the toxin is added in instalments, the resulting mixture is toxic. This phenomenon is exactly analogous to what happens in the precipitation of a colloid by an electrolyte, or in the precipitation of one colloid by another; the amount of electrolyte or colloid required for complete precipitation varies according as it is added all at once or in instalments. The condition of a toxin-antitoxin mixture, therefore, resembles that of colloidal solutions in that it is not completely defined by a statement of its composition; its character depends on its previous history.

In connection also with the agglutination of bacteria,[1] notable attempts have been made to interpret some at least of the phenomena by reference to the known behaviour of ordinary colloids.[2] If an animal is inoculated with cultures of typhoid bacteria, a substance, agglutinin, is produced in the serum, and this substance, when added to a suspension of typhoid bacteria, causes the latter to clump together and to sink to the bottom of the liquid in which they are suspended: this phenomenon is described as 'agglutination.' This process bears a general resemblance to the precipitation of an insoluble salt which frequently follows the addition of one salt solution to another, but to conclude from this that the interaction between typhoid bacteria and agglutinin is purely a chemical reaction would be unjustifiable. For, as we have seen, a colloid may be precipitated by an electrolyte or by another colloid, even in cases where the possibility of chemical interaction in the ordinary sense is excluded.

When different quantities of agglutinin are added to a given quantity of typhoid bacteria, it is found that for one particular quantity of agglutinin the agglutination is at a maximum. If either a very small or a very large amount of agglutinin is added to the bacteria, no agglutination whatever occurs. The occurrence of such maximum effects for particular concentrations of the interacting substances is indeed fairly frequent in the field of immunity. It is possible to regard this phenomenon as the analogue of what happens when sodium hydroxide is gradually added to a solution of alum; the precipitate first formed is dissolved by excess of the reagent, and the amount of precipitate is a maximum for certain definite proportions of alum and sodium hydroxide. But the interaction between agglutinin and

[1] Eisenberg and Volk, *Zeit. Hygiene*, 1902, 40, 155.

[2] Bechhold, *Zeit. physikal. Chem.*, 1904, 48, 385; Biltz, *ibid.*, 615.

bacteria is not thereby proved to be purely a chemical effect, for, as already stated (p. 209), the mutual precipitation of colloids is characterised by the same features. When one of the colloids is in very large excess no precipitate is formed, and the maximum precipitation for a given quantity of the one colloid is obtained only with a certain proportion of the other colloid.

It is noteworthy that a serum containing agglutinin can agglutinate bacteria only in the presence of the salts of the serum; if the serum is dialysed, and so freed from electrolytes, no agglutination takes place. That this should be so is not surprising when we bear in mind the important part played by electrolytes in relation to colloids. A suspension of agglutinin bacteria—that is, bacteria which have been treated with a serum containing agglutinin and thereafter thoroughly washed—resembles a mastic suspension in being completely precipitated by small quantities of salts, and Bechhold (*loc. cit.*) has shown that the precipitating power of a salt in relation also to agglutinin bacteria is determined mainly by the valency of the cation. A suspension of typhoid bacteria alone, although it moves towards the anode in a potential gradient, is not precipitated by sodium chloride. The bacteria behave like suspended particles which are provided with a coating of reversible colloid, and are so protected from the action of salts of the alkali and alkaline earth metals.

Such are a few of the cases in which a comparison of the agglutination of bacteria and the properties of ordinary colloids is highly suggestive. The problem of agglutination, however, is very complex, and it is unlikely that it will be solved merely by correlation with the phenomena of colloidal solutions, or even on the wider basis of an adsorption theory (to be discussed in the following chapter). For the reaction between agglutinin

and bacteria is a specific one; typhoid bacteria are agglutinated pre-eminently by the serum of animals previously treated with cultures of typhoid bacteria, not by the serum of animals inoculated with cultures of other bacteria. Such specific characteristics can be explained only on chemical lines, and a discussion of the agglutination of bacteria from this point of view is outside the scope of the present volume.

There are various other phenomena of physiological interest in which the mutual precipitation of two colloids is a main feature, and in the interpretation of which the conditions governing such a precipitation must be borne in mind. There is, for instance, the observation that if red blood corpuscles from one animal are injected into another animal of a different species, a substance is produced in the serum of this second animal which has the power of agglutinating red corpuscles of the injected variety. In this connection it is worthy of mention that when blood corpuscles are suspended in a sucrose solution, or in a neutral solution of an alkali or alkaline earth salt, they move towards the anode in a potential gradient. Höber[1] attributes this to the protein and lecithin present in the plasmatic membrane; these substances generally exhibit anodic convection. Like these also, the blood corpuscles reverse their migration when a little acid, copper, silver, iron, or aluminium salt is added to the medium in which they are suspended. A suspension of red blood corpuscles is agglutinated not only by serum obtained from an animal which has been inoculated with these corpuscles, but also by numerous colloids, positive as well as negative—stannic acid, ferric hydroxide, mastic, and various dyes.

[1] *Pflüger's Arch.*, 1904, 101, 607; 102, 196.

CHAPTER XI

ADSORPTION

Surface Development in Colloids. — It is not proposed to discuss in detail in this volume the various theories which have been brought forward dealing with the stability of colloidal solutions and with the separation of colloids from their solutions.[1] Two factors, however, which must obviously enter into any interpretation of the relation between a colloid and its medium may be noted here. There is, firstly, the existence in a great many cases at least of a potential difference between the colloid particles and the surrounding medium, and secondly, the relatively enormous surface of contact between the colloid and its environment. The importance of the electrical factor will have become plain to the reader from the facts described in the two preceding chapters. A little consideration will show that the other factor, which we may call the surface factor, is equally important in the interpretation of the phenomena exhibited by colloids. All the facts go to show that a colloidal solution is essentially non-homogeneous; it is what is known as a two-phase system, built up of a fluid medium containing definite and distinct suspended particles in an extreme state of subdivision. With the help of the ultramicroscope it is possible in

[1] See, for instance, Hardy, *Zeit. physikal. Chem.*, 1900, 33, 385; Bredig, *Anorgan. Fermente*, Leipzig, 1901; Billitzer, *Zeit. physikal. Chem.*, 1904, 45, 327; 1905, 51, 129; Michaelis, in Koranyi and Richter's *Physikalische Chemie und Medizin*, Leipzig, 1908.

a great many cases to detect these particles and to follow their movements. Now, when a given quantity of matter is divided up more and more finely, its surface area is immensely increased. Suppose, for instance, that a compact sphere of any material with a diameter of 1 mm., and therefore a surface area of 0·0314 sq. cm., were divided up into a number of small spheres each with a diameter of 0·01 mm. The number of spheres would now be 10^6, and the total area of their surfaces would be 3·14 sq. cm. If the division were carried farther until each small sphere had a diameter of 0·0001 mm., and would therefore be hardly visible under the microscope, their number would be 10^{12}, and the total area of their surfaces would be 314 sq. cm. To bring about such a subdivision requires the application of energy, which is stored up in the finely divided spheres in the form of surface energy; this is defined as the product – surface area × surface tension. In two-phase systems, therefore, where the surface of the one phase is developed to a relatively high degree, as it is in colloidal solutions, the surface energy becomes an important factor in determining the behaviour of the system.[1] Especially is this the case where a change in the aggregation of colloid particles is concerned; this means a change in the surface area, and this again involves a change of the surface energy.

It has been stated above that in the relationship between a colloid and its environment electrical energy also is involved. The parts played by electrical and surface energy respectively in the phenomena of colloidal solutions are not however independent of each other: there is a close connection between the two. It is

[1] It is an interesting question whether this argument cannot be extended to cover the case of crystalloidal solutions, regarded as two-phase systems. See Wo. Ostwald, *Grundriss der Kolloidchemie*, p. 126.

well known that the surface tension of mercury in contact with a sulphuric acid solution is affected by alterations in the potential difference between metal and solution. Obviously, in view of the fact that electrical charges of the same sign repel each other, the existence of a charge at the surface of the mercury tends to increase the surface, and is therefore opposed to the surface tension, which tends to diminish the surface. A reduction in the electrical charge at the surface means an increase in the surface tension. This example shows that in the case of colloidal solutions the connection between the electrical and surface factors must be very close.

Instead of following out the influence of these factors in determining the properties of colloids, as has been attempted by Hardy, Bredig, Billitzer, and others, we shall consider the phenomena exhibited by colloids from a wider point of view which has been very generally adopted in recent years. We may regard the interaction of colloids with each other and with various solid and dissolved substances as being essentially a process of 'adsorption.' This term is used to describe a phenomenon which is frequently observed when a foreign substance is introduced into a two-phase system. When opportunity is afforded this foreign substance to distribute itself throughout the system, it is often found to be locally concentrated at the surface of one of the phases. This concentration, as will appear from the cases discussed below, is not generally to be regarded as a chemical process; the phenomenon is physical in character, and is especially striking when the surface of the adsorbing phase is highly developed. It is in regard to this local concentration on the surface that adsorption differs from absorption; when we speak of a gas as being absorbed by a liquid, we picture the gas as distributed uniformly throughout the mass of the liquid. Perhaps, however, we can best

appreciate what is involved in the term 'adsorption' by studying, first, the distribution of a substance in a two-phase system where no surface concentration occurs.

Distribution of a Substance between Two Immiscible Liquids.—When a substance is shaken up with two immiscible liquids, some of it is found to be dissolved in the one layer, some of it in the other layer; it is said to be 'distributed' between the two phases. The absolute amount of the substance found in each liquid layer after equilibrium has been established will naturally depend on the volume of each liquid taken, but if we eliminate this by comparing the *concentrations* (*i.e.* weights per unit volume) of the substance in the two layers, we get a definite measure of the distribution. If c_1 is the concentration of the substance in the first liquid, and c_2 its concentration in the second liquid, then the ratio $\frac{c_2}{c_1}$ is known as the 'partition coefficient' or the 'distribution ratio.' Experiment has shown that if the molecular condition of the dissolved substance is the same in each of the two liquids, then the partition coefficient is independent of the absolute values of c_1 and c_2, independent, in other words, of the concentration. This is illustrated by the figures in the following table relating to the distribution of iodine between water and carbon tetrachloride;[1] the figures in the first column (c_1) are the concentrations of iodine in the aqueous layers, those in the second column are the concentrations of iodine in the corresponding carbon tetrachloride layers:—

c_1.	c_2.	$\frac{c_2}{c_1}$.
0·2913	25·61	87·9
0·1934	16·54	85·5
0·1276	10·88	85·3
0·0818	6·966	85·1
0·0516	4·412	85·8

[1] Jakowkin, *Zeit. physikal. Chem.*, 1895, **18**, 585.

The rule, of which the foregoing figures are an illustration, namely, that the ratio of the concentrations of a substance distributed in two immiscible liquids is independent of the concentration, is really the same as Henry's law dealing with the absorption of a gas by a liquid under varying pressures. In an earlier part of this volume (p. 20) it was stated that according to Henry's law the quantity of gas dissolved by a given quantity of a liquid at a given temperature is proportional to the pressure. Suppose that a given quantity of water is shaken up with hydrogen (1) at 1 atmosphere pressure, (2) at 3 atmospheres pressure, until saturation is complete in each case, that is, until equilibrium is established between the gas phase and the liquid phase. According to Henry's law, the quantity of gas dissolved in the liquid in the second case is three times as great as it is in the first case, that is, its concentration in the liquid phase is three times as great. But the hydrogen in the second case is under 3 atmospheres pressure as compared with 1 atmosphere in the first case, and the concentration in the gas phase will have increased in the same proportion. The *ratio*, therefore, of the concentrations of hydrogen in the gas phase and in the water is the same under 3 atmospheres as under 1 atmosphere; or, putting it generally, the ratio of the concentrations of the gas in the gas phase and in the liquid phase, when equilibrium has been established, is independent of the pressure. Expressed in this form, Henry's law is seen to be practically identical with the rule relating to the distribution of a substance between two immiscible liquids.

Such a distribution is a physical process; it can be regarded as chemical only in so far as we regard the process of solution of, say, sucrose in water as due to

the action of chemical forces. It ought to be noted also that when a gas is dissolved in a liquid, or when a substance is distributed between two immiscible liquids, the equilibrium which is established is reversible, that is, it can be reached from both sides. Suppose, for instance, that 100 cub. cm. of water are shaken with 20 cub. cm. of a solution of iodine in carbon tetrachloride; equilibrium is rapidly established, when it will be found that some of the iodine has gone into the water. Suppose that, in a second experiment, 100 cub. cm. of water are shaken with 10 cub. cm. of an iodine solution of double the concentration of the first one. When equilibrium is reached the 100 cub. cm. of water will be found to contain more iodine than in the first case. If, however, another 10 cub. cm. of carbon tetrachloride are added and the mixture is shaken, the extra iodine is taken out of the water, and the equilibrium finally reached is the same as in the first case.

If the molecular condition of the dissolved substance is different in the two liquids, then the value of the ratio $\frac{c_2}{c_1}$ is not independent of the concentration. This statement is borne out by the following figures relating to the partition of acetic acid between benzene and water:[1]—c_1 is the concentration of the acid in the benzene layer, c_2 the concentration in the aqueous layer.

c_1.	c_2.	$\frac{c_2}{c_1}$.	$\frac{c_2^2}{c_1}$.
0·043	0·245	5·7	1·40
0·071	0·314	4·4	1·39
0·094	0·375	4·0	1·49
0·149	0·500	3·4	1·67

It is obvious that the partition coefficient varies with the concentration, and this is to be attributed to the fact

[1] Nernst, *Zeit. physikal. Chem.*, 1891, 8, 110.

that the molecular condition of acetic acid is not the same in benzene as it is in water. From the depression of the freezing point produced by acetic acid in these two solvents, it is known that the acid in benzene solution consists almost entirely of double molecules (although the proportion of simple molecules increases with dilution), whereas acetic acid in water, apart from the slight electrolytic dissociation, exists in the form of simple molecules. Now, on theoretical grounds it follows that if the molecular weight of the dissolved substance in the first liquid is n times the molecular weight in the second liquid, then the ratio $\frac{c_2^n}{c_1}$ ought to have a constant value independent of the concentration. From what has been said, it is evident that for the partition of acetic acid between benzene and water $n=2$, and we should therefore expect the value of $\frac{c_2^2}{c_1}$ to be independent of the concentration.[1] This is approximately the case, as shown by the figures in the last column of the foregoing table, and such variation as the figures show is probably due to the fact that the proportion of simple molecules in a benzene solution of acetic acid increases somewhat on dilution.

If, conversely, the distribution of a substance between two liquids at various concentrations has been found to be such that $\frac{c_2^n}{c_1}$ is independent of the concentration, the conclusion may be drawn that the molecular weight of the substance in the first liquid is n times that in the second liquid.

Equilibrium between a Gas and a Solid.—As an example of a case where surface effects become prominent, we may take, first, the distribution of a gas

[1] Nernst, *loc. cit.*

between a gas phase and a solid phase; that is, we shall consider the way in which the amount of a gas taken up by a solid varies with the pressure of the gas. In view of the results obtained in connection with the distribution of a substance between two non-miscible liquids, it might be expected that a study of the equilibrium between a gas and a solid would lead to a knowledge of the molecular condition of the gas which is taken up by the solid. This expectation, however, is not fulfilled, for the taking up of a gas by a solid is found to be determined mainly by surface effects.

The facts can best be explained by reference to the case of carbon dioxide and carbon, studied by Travers.[1] This investigator determined the concentration (x) of carbon dioxide in the carbon at various pressures (P), sufficient time of course being allowed for the gas and solid to come into equilibrium with each other. The results obtained at 0° C. are recorded in the first two columns of the following table:—

P mm.	x.	$\frac{x}{\sqrt[3]{P}}$.
4·1	0·38	0·24
25·1	0·77	0·26
137·4	1·45	0·26
416·4	2·02	0·27
858·6	2·48	0·26

From a consideration of these it is seen that the amount of carbon dioxide taken up by the carbon increases much more slowly than the pressure. Travers found, however, that x increases proportionally to the cube root of P, as is shown by the constancy of the figures in the third column of the table, and the question arises: What interpretation is to be given of this relationship between

[1] *Proc. Roy. Soc.*, A, 1906, 78, 9. Compare Homfray, *ibid.*, 1910, 84, 99.

P and x? How is it that a similar relationship, expressed by the equation $\frac{x}{\sqrt[n]{P}} = \text{const}$, is found for carbon dioxide and carbon at other temperatures, as well as for hydrogen and carbon? If we suppose that the gas is uniformly distributed throughout the carbon, forming a solid solution, and reason by analogy from Nernst's experiments on the partition of acetic acid between water and benzene, we should reach the conclusion that the molecular weight of carbon dioxide in carbon is one-third of its molecular weight in the gaseous condition. It is obvious that on chemical grounds this conclusion must be rejected, and it appears therefore that to regard the carbon dioxide as uniformly distributed through the carbon, and so forming a homogeneous solid solution, is not permissible. There are various indications, in this and similar cases, that the *surface* of the solid is mainly, if not exclusively, concerned in taking up the gas, and the phenomenon is accordingly described as 'adsorption' rather than 'absorption.'

Adsorption by a Solid from a Solution.—Very similar to the phenomena just discussed is the power of carbon to adsorb various substances from their solutions. Numerous cases of this adsorption have recently been investigated with the object of discovering the nature of the equilibrium between the carbon and the solution, and of finding how the quantity of substance adsorbed by the carbon varies with its concentration in the solution.

It is noteworthy that the adsorption equilibria between carbon and an aqueous solution are reversible—that is, they can be reached from either side. An illustration of this important point may be quoted.[1] One gram of

[1] Freundlich, *Zeit. physikal. Chem.*, 1906, 57, 385.

carbon was shaken for 20·5 hours with 100 cub. cm. of a 0·0688N solution of acetic acid; by this time equilibrium was established, and it was found that the acid solution was now 0·0608N. In another experiment one gram of the same carbon was shaken for 21 hours with 50 cub. cm. of a $0{\cdot}1376N(=0{\cdot}0688\times 2)$ solution of acetic acid; 50 cub. cm. of water were then added, and the mixture was shaken for an hour, at the end of which time it was found that the acid solution was 0·0606N. This is practically the same value as in the first case, which shows that the same equilibrium is reached when the carbon is charged directly with the acid as when a slightly overcharged carbon is deprived of part of its adsorbed acid.

In the experiments which have just been described the carbon was shaken with the acid for about 20 hours in order to secure the establishment of equilibrium. In reality, however, the time required is remarkably short. It has been found that when a solution of acetic acid is added to carbon, once shaken with the hand, and then allowed to stand for 20 minutes, the concentration of the solution has fallen very nearly to its equilibrium value. This observation supports the view that the taking up of acetic acid from its solutions by carbon is a process in which the *surface* of the carbon is mainly concerned, for the penetration or diffusion of the acid into the interior of the carbon granules could only be a comparatively slow process.

The relation between the concentration of acetic acid in the carbon and that in the solution when equilibrium has been reached is brought out by the figures in the following table.[1] Those in the first column (c_l) represent the equilibrium concentrations of the acetic acid

[1] Freundlich, *loc. cit.*

in the solutions, and are given in millimolecules per cub. cm.; the figures in the second column (c_s observed) represent the equilibrium concentrations of the acetic acid in the carbon, and are given in millimolecules per 1 gram of carbon. A glance at the table shows that the amount of acetic acid taken up by the carbon increases much more slowly than its concentration in the solution. In this respect the adsorption of dissolved acetic acid by carbon and the adsorption of carbon dioxide by the same substance are closely similar.

Adsorption of Acetic Acid by Carbon.

$\beta = 2{\cdot}606.$ $p = 2{\cdot}35.$

c_l.	c_s observed.	c_s calculated.
0·0181	0·467	0·474
0·0309	0·624	0·596
0·0616	0·801	0·798
0·1259	1·11	1·08
0·2677	1·55	1·49
0·4711	2·04	1·89
0·8817	2·48	2·47
2·785	3·76	4·01

The parallelism, however, goes further, for the relationship between the concentrations of acetic acid in the solution and in the carbon can be represented by a formula of the same general type as $\frac{x}{\sqrt[y]{P}} = \text{const.}$ This general adsorption formula is $c_s = \beta . c_l^{\frac{1}{p}}$, in which β and p are constants for a given temperature and a given dissolved substance, while c_s and c_l represent, as already stated, the concentration of the dissolved substance in the solid and liquid phase respectively.[1] The applicability of this formula to the adsorption of acetic acid by carbon may be tested by assuming that the formula $c_s = \beta . c_l^{\frac{1}{p}}$ is valid in this case, and then using the experimental figures of the first two columns to evaluate β and p. The mean values so

[1] For other formulæ see Arrhenius, *Medd. K. Vetensk. Nobelinst.*, 1911, **2**, No. 7, 1.

obtained are $\beta = 2{\cdot}606$ and $p = 2{\cdot}35$. When these figures are put in the general formula, we get $c_s = 2{\cdot}606 c_l^{\frac{1}{2{\cdot}35}}$, so that from the ascertained value of c_l, given in the first column, we can calculate what the value of c_s ought to be. Agreement between the value of c_s so calculated and the experimental value of c_s furnishes a proof of the applicability of the original exponential formula. The numbers in the third column of the foregoing table are the calculated values of c_s for each solution, and it will be seen that they agree remarkably well with the observed values.

The empirical exponential formula, then, $c_s = \beta . c^{\frac{1}{p}}$, may be taken as representing the adsorption equilibrium between carbon and aqueous acetic acid. Freundlich has further shown that the same general formula is applicable to the adsorption of many other substances by carbon, not only from their aqueous solutions, but also from their solutions in alcohol, benzene, and ether. The value of p varies from one case to another, but only within somewhat narrow limits; it appears, therefore, to be to a large extent independent of the solvent and the dissolved substance. Thus for benzoic acid in water $p = 2{\cdot}96$; bromine in water, $p = 3{\cdot}44$; picric acid in water, $p = 4{\cdot}17$; benzoic acid in ether, $p = 2{\cdot}2$. The similarity between the adsorption of two such different substances as benzoic acid and bromine, evidenced by the comparatively slight difference in the values of p, is particularly striking,[1] and may be taken as showing that the process of adsorption is not generally a chemical phenomenon in the ordinary sense, as has been maintained in some quarters.

Further, when a solution of a substance is shaken up with a solid with which it may react chemically, the equilibrium reached is essentially different from the

[1] See Freundlich, *Zeit. Chem. Ind. Koll.*, 1908, 3, 212.

adsorption equilibrium between, say, carbon and aqueous acetic acid. The difference is well brought out in an investigation by Walker and Appleyard[1] of the equilibrium between solid diphenylamine and an aqueous solution of picric acid. Diphenylamine unites with picric acid to form a compound, diphenylammonium picrate, and both the amine itself and the compound are practically insoluble in water. The compound is capable of dissociating into its constituents, for when it is treated with water some of the picric acid dissolves, and an equivalent quantity of diphenylamine remains behind; if the treatment with water is continued long enough, all the picric acid is extracted from the compound. In their experiments Walker and Appleyard shook three lots of 50 cub. cm. of saturated picric acid solution (=16·8 mg. acid per gram of water at 40·6°) with 2 grams, 1 gram, and 0·5 gram of diphenylamine for 4½ hours, and then, equilibrium having been reached, determined the concentrations of the picric acid in the water and in the diphenylamine. The results for the three experiments are shown in the adjoining table:—

Milligrams of Picric Acid.	
In 1 gram Water.	In 1 gram Diphenylamine.
13·8	7·5
13·7	15·5
13·8	30·0

It is obvious that the equilibrium concentration of the picric acid in the diphenylamine has risen steadily, while that in the solution has remained constant.

These results are quite distinct from the adsorption phenomena already discussed, and show how the distribution of a substance between a liquid and a solid phase is affected by the intervention of chemical affinity. This is an important point, and it may be well to

[1] *Journ. Chem. Soc.*, 1896, 69, 1334.

indicate graphically the distinction between adsorption and chemical combination; this may be done by tracing in each case the curve which represents the corresponding variations of c_s and c_l. Suppose, in the first place, that we have a case of pure adsorption, to which the exponential formula is applicable. Such a case is represented by the continuous curve 1, which is concave to the c_l axis. Its course is obviously in harmony with the observation which is made in all cases of adsorption, namely, that c_s increases much more slowly than

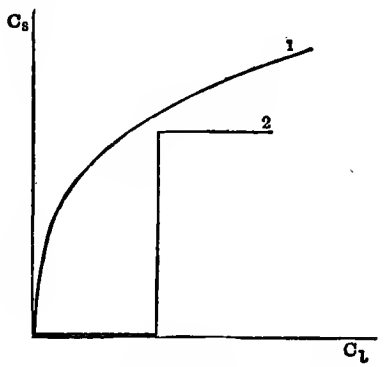

FIG. 23.

c_l; for very small values of c_l the adsorption is relatively complete. In a case of chemical combination, on the other hand, none of the dissolved substance is taken up by the solid until its concentration in the solution reaches a certain value (13·8 mg. picric acid per gram of water in the case studied by Walker and Appleyard); up to this point, that is, c_l increases steadily, while c_s remains equal to zero. When the critical value is reached, however, any attempt to increase c_l further results merely in an increase of c_s, while c_l remains constant. This continues until the formation of the

compound is complete, when c_l may increase again. The curve 2, therefore, representing the variation of c_s and c_l in the case where the solid forms a compound with the dissolved substance, is simply a broken line, the vertical part of which corresponds with the interval over which formation of the compound is proceeding.

In view of the distinction which has just been drawn, it is fairly clear that the fixation of, say, acetic acid by carbon is not due to any chemical interaction in the ordinary sense. The suggestion that acetic acid forms a solid solution in carbon must also be rejected, for in this case we should have to conclude from the observed recorded data that the molecular weight of acetic acid dissolved in carbon is less than half its molecular weight in water. This is not in the least credible. At the same time it must be allowed that in certain other cases there is evidence for the formation of a solid solution in addition to surface adsorption. Thus Davis has shown,[1] in a study of the equilibrium between carbon and a solution of iodine in various organic solvents, that when the carbon is brought into contact with an iodine solution there is, first, a surface condensation, which is complete in a few hours, followed by a slow diffusion into the mass of the carbon; this latter process goes on for weeks or months. In experiments carried on for only a short time, the same equilibrium point is reached from both sides, but the amount of iodine contained in the carbon under such equilibrium conditions is much less than the amount which it takes up after prolonged contact with the iodine solution.

In the cases of adsorption which have been cited so far, there can be no doubt that surface condensation

[1] *Journ. Chem. Soc.*, 1907, 91, 1666. See also McBain, *Zeit. physikal. Chem.*, 1909, 68, 471.

plays the main part, and indeed it appears that on thermodynamical grounds the most stable condition of any solution, when surface tension considerations only are taken into account, is the one characterised by a minimum surface tension. Hence if the solute lowers the surface tension of the solvent, it will accumulate in the surface layer of the solution. Such spontaneous accumulations in surface layers are well known. Ramsden has shown[1] that solid or highly viscous coatings are formed on the free surfaces of protein solutions, of other colloidal solutions, of fine and coarse suspensions, and of a few apparently crystalloidal solutions. A similar interpretation can be offered of the local concentration which occurs at the common surface of adsorbing solid and solution.

Noteworthy in this connection are the views of Macallum,[2] who contends that surface tension is a prime factor in such vital phenomena as muscular contraction, secretion and excretion, and cell division, and traces differences in the surface tension of living matter by a microchemical study of the distribution of inorganic salts.

Adsorption of Arsenious Acid by Ferric Hydroxide.—We may now proceed to consider cases of adsorption which are complicated by the irreversibility of the equilibrium or the intervention of chemical affinity. One interesting case where chemical affinity may be a factor, is the use of freshly precipitated ferric hydroxide as an antidote in cases of arsenical poisoning.

The power of ferric hydroxide to remove arsenious acid from its solutions has generally been attributed to the formation of a basic ferric arsenite, but Biltz has found[3] that it is a typical case of adsorption.

[1] *Proc. Roy. Soc.*, A, 1903, 72, 156.

[2] *Brit. Assoc. Report*, 1910, 740; *Proc. Roy. Soc.*, B, 1913, 86, 527.

[3] *Ber. deut. chem. Ges.*, 1904, 37, 3138.

When the hydroxide is shaken with a solution of arsenious acid, the equilibrium which is established is reversible, for it can be reached from both sides. Experiments in which a definite quantity of freshly precipitated ferric hydroxide was shaken with a definite volume of solution containing different amounts of arsenious acid showed that if x is the equilibrium concentration of arsenious acid in the solution, and y the corresponding concentration in the hydroxide, then the observations are in harmony with the formula $y = Kx^{\frac{1}{5}}$. That is, the distribution of arsenious acid between water and precipitated ferric hydroxide is essentially the same as the distribution of acetic acid between water and carbon; in both cases the removal of the dissolved substance from the solution is relatively complete in very dilute solution. This analogy makes it very unlikely that the removal of arsenious acid from solution by ferric hydroxide is due to the formation of a compound, and the fact that arsenious acid in aqueous solution has a normal molecular weight makes it impossible to regard the process as due to the formation of a solid solution. Regarding it, on the other hand, as a case of surface condensation, we can understand why the efficiency of ferric hydroxide in removing arsenious acid from solution depends on its physical condition.

Adsorption of Dyes.—The adsorbed substances so far considered have been crystalloids. Many of the most interesting cases of adsorption, however, are furnished by organic dyes, some of which in aqueous solution are crystalloid in character, while others are colloidal. Walker and Appleyard[1] were able to show that when silk is dyed with picric acid a real equilibrium is attained

[1] *Journ. Chem. Soc.*, 1896, 69, 1334.

which is independent of the original distribution; that is, the equilibrium is reversible. They showed also that, if s represents the equilibrium concentration of picric acid in the silk, and w the corresponding concentration in the water, the experimental results are satisfactorily reproduced by the formula $s=Kw^{\frac{1}{p}}$, the usual adsorption formula.

More recently it has been found[1] that the same general formula represents the adsorption of crystal violet and patent blue by carbon, of crystal violet and patent blue by wool, of new magenta and patent blue by silk, and of crystal violet and new magenta by cotton. In all these cases the adsorption equilibrium is reversible, and it is remarkable that the values of p (exclusive of the cases in which crystal violet was used) all lie between 4 and 7·7.

So far, then, as these experiments go, they give support to the view that the process of dyeing is essentially an adsorption phenomenon, not depending on any chemical affinity between the dye and the fibre, or consisting in the formation of a solid solution in the fibre. There are, however, a number of observations which indicate that other factors besides adsorption have to be taken into account in interpreting the relation of a dye to the fibre. The very fact that it is possible to get a fast colour in certain cases proves that the equilibrium between dye and fibre is not always reversible. In such cases one might be inclined to regard the process as a chemical interaction resulting in the production of an insoluble compound of the nature of a salt. The view of dyeing as a chemical reaction appears to be supported also by the observation that when a basic colouring matter (that is, an organic base + an inorganic acid, for example,

[1] Freundlich and Losev, *Zeit. physikal. Chem.*, 1907, 59, 284.

magenta or crystal violet) is employed to dye wool, the base alone is taken up by the fibre, while the acid is left in the solution. In spite of this, the colour of the dyed fibre is that of the salts of the base. All this is very suggestive of chemical action, but the curious thing is that a similar splitting up of the colouring matter into base + acid occurs when carbon or pure cellulose is used in place of wool. In these cases the suggestion of salt-formation cannot be accepted. Altogether the problem is a very complicated one, and cannot be fully discussed here. It appears very probable that the splitting up of the basic dyes which has just been mentioned is an electrical phenomenon, and that the adsorption pure and simple is masked to some extent.[1] The non-reversible character of the equilibrium between dye and fibre in some cases may be attributed to the transformation of the free base deposited on the fibre into a tautomeric modification.[2]

That both adsorption and chemical action may be concerned in a dyeing process is shown by an observation recorded by Bayliss.[3] If well-washed aluminium hydroxide is added to a dilute solution of the blue colloidal free acid derived from congo red, the dye is taken up by the suspended hydroxide, which is coloured blue. If this blue product is suspended in water and warmed, chemical union takes place, and the aluminium salt of congo red is formed, which has the usual red colour of the salts.

Attention has already been drawn to the interesting fact that when a basic colouring matter is employed to dye wool the free base alone is taken up by the fibre,

[1] See Michaelis in Koranyi and Richter's *Physikalische Chemie und Medizin*, vol. ii. p. 350.

[2] See Freundlich and Losev, *loc. cit.*, p. 301.

[3] *Zeit. Chem. Ind. Koll.*, 1908, 3, 224.

while the acid remains in solution. The fibre, in fact, decomposes the dye-salt into acid and base. Other cases where colloids have this effect are known. In the foregoing chapter it was pointed out that when a colloidal solution of arsenious sulphide is coagulated by the addition of barium chloride some of the salt is decomposed; the coagulum is found to contain barium, while the solution contains an equivalent quantity of hydrochloric acid. In both these cases, as in many others, the electrical charge on the solid phase is the main factor in determining the surface equilibrium.

Proteins and Adsorption.—The previous pages will have shown how very common is the phenomenon of adsorption. To sum up: The equilibrium between a solid phase and a solution in which it is immersed is frequently characterised by a local concentration of the dissolved substance at the surface of the solid phase, and in the majority of cases it is impossible to interpret this phenomenon by assuming the formation of a solid solution or the occurrence of a chemical reaction between solid and dissolved substance. The equilibrium is therefore described as an adsorption equilibrium, and one of its most prominent characteristics is the fact that the amount of dissolved substance taken up by the solid increases much more slowly than the concentration of the solution; the removal of the dissolved substance is therefore relatively most complete in dilute solution. These facts find a definite expression in the adsorption formula: $c_s = \beta \cdot c_l^{\frac{1}{p}}$ where $p > 1$.

The magnitude of the adsorption effect will of course depend on the surface development of the adsorbing solid. Hence it is that carbon in the form of charcoal has been so largely used in the study of adsorption. But in any case where the surface area of a solid is relatively great

for its volume, the conditions are favourable for the phenomenon of surface condensation. Whether such a solid immersed in a solution will exhibit the phenomenon will naturally depend to some extent on the nature of the dissolved substance, and especially on its electrical character. Since surface development is a preliminary condition for the manifestation of adsorption, and since that condition, according to the argument at the beginning of this chapter, is satisfied by colloidally dissolved substances, it is not surprising that the behaviour of colloids is capable in many cases of being referred to the occurrence of adsorption phenomena. The opinion has rapidly gained ground that where mixtures of colloids and ions are involved, as in the living cell, the equilibrium between these partakes largely of the nature of an adsorption equilibrium. In such a complex case it is, of course, impossible to say exactly what part is played by the chemical and physical factors respectively, but the study of proteins is showing that these substances, which are so essentially associated with the living cell, are peculiarly liable to exhibit adsorption phenomena. Not only are proteins readily adsorbed by charcoal, mastic suspension, kaolin suspension, and freshly precipitated ferric hydroxide, but they themselves appear to adsorb electrolytes from solution.

There are many grounds for the conclusion that the experimental behaviour of proteins is best interpreted in terms of adsorption. One of the lines of investigation which lead up to this view may be briefly sketched here. The temperature of heat coagulation of protein, as recently shown by Pauli,[1] is markedly affected by traces of salts. The protein solution used by this investigator was obtained by long-continued dialysis of ox-blood serum, and exhibited no migration in an electric field;

[1] *Zeit. Chem. Ind. Koll.*, 1908, 3, 2.

the protein was therefore electrically neutral. When neutral salts of the alkali or alkaline earth metals are added in very small quantity to such a protein solution, so that the concentration of the salt in the mixture is not above 0·05N, the temperature of heat coagulation of the protein is raised in all cases. The lower the concentration of the salt solution, the greater relatively is the extent to which the coagulation is inhibited. If t_0 is the temperature of heat coagulation for the protein solution alone, t that for the solution of protein + salt, and c is the concentration of the added salt, then it can be shown that the experimental data are in harmony with the formula $t - t_0 = Kc^m$, $m < 1$, which is obviously of the same type as the ordinary adsorption formula. Since the inhibiting effect is not produced by non-electrolytes, and since the effect is relatively most marked in dilute solution, Pauli concludes that there is an adsorption equilibrium between the protein particles and the ions of the salt, and that the change thus brought about in the surface of the protein particles is such as to hinder their further aggregation.

Agglutination as an Adsorption Phenomenon.—One of the most interesting cases in which the adsorption formula is found to be applicable is the agglutination of bacteria by immune serum. As already stated, if the serum of an animal which has previously been injected with typhoid bacteria is added to a suspension of these bacteria, the latter clump together and settle to the bottom of the containing vessel. On examination the bacteria are found to have taken up a certain part of the agglutinin contained in the serum, and it is an interesting question what is the relation between the amount of agglutinin taken up by the bacteria and the amount which remains in solution. The problem

was attacked by Eisenberg and Volk,[1] who added a given volume of agglutinin solutions of different concentrations (obtained by diluting the serum with physiological salt solution) to equal quantities of suspensions of typhoid bacteria. Equilibrium is reached very rapidly, and the distribution of the agglutinin is ascertained by centrifuging and then examining the clear liquid; a measure of the agglutinin left in this clear liquid is obtained by finding the extent to which it must be diluted with physiological salt solution before it ceases to produce agglutination under given conditions. In this way a uniform measure is obtained for the original agglutinin solution added to the bacteria, and for the solution which has come into equilibrium with the bacteria.

The results obtained by Eisenberg and Volk for the agglutination of typhoid bacteria by agglutinin are recorded in the first three columns of the following table. The figures given under T represent the quantities of agglutinin (in arbitrary units) added to the bacteria, while those under S (obs.) represent the quantities of agglutinin left in the solutions after the agglutination of the bacteria. $B = T - S$ (obs.) is the quantity of agglutinin taken up in each case by the bacteria; the figures under S (calc.) are obtained in a manner to be described presently.

T.	B.	S (obs.).	S (calc.).
2	2	0	0·02
20	20	0	0·7
40	40	0	2·1
200	180	20	19·7
400	340	60	52·9
2,000	1,500	500	478
10,000	6,500	3,500	3,890
20,000	11,000	9,000	9,160

These figures bring out a feature which we found to

[1] *Zeit. Hygiene*, 1902, 40, 155.

be characteristic of adsorption, viz. the more dilute the solution, the more completely is the dissolved substance taken up by the solid phase. More than that, the relation betwen B and S is satisfactorily represented by the formula $B=KS^{\frac{1}{3}}$, where $K=24{\cdot}7$ for the whole series. The figures given in the last column of the table have been obtained by taking the numerical value of B in each case, and calculating S by means of the formula. The errors of observation are considerable, and it is stated that it is impossible to determine values of S below 1. In these circumstances the agreement between the observed and calculated values of S is satisfactory, and permits the conclusion that the equilibrium between bacteria and agglutinin may fairly be represented by a formula of the ordinary adsorption type.[1]

The applicability of the foregoing formula to the distribution of agglutinin between typhoid bacteria and agglutinin solution was first demonstrated by Arrhenius, who however rejects the adsorption theory, and maintains that the agglutination is not to be attributed to a special surface action. He believes that the bacterial cell contains a substance which is a good solvent for the agglutinin, and draws the conclusion that the molecular weight of the agglutinin in this solvent is two-thirds of the molecular weight of the agglutinin in the surrounding fluid.[2]

It is doubtful whether it is permissible to regard the applicability of the empirical adsorption formula as definitely establishing the nature of the equilibrium between bacteria and agglutinin. The substances involved are complex, and there are one or two facts which suggest caution. There is, firstly, the specificity of the agglutinins; that is, the agglutinin produced by

[1] See Craw, *Journ. Hygiene*, 1905, 5, 113.

[2] See *Immunochemistry*, p. 148.

injecting an animal with typhoid bacteria is capable of agglutinating typhoid bacteria in a pre-eminent degree. Such a fact suggests that agglutination may be something more than a purely physical phenomenon. Secondly, there is evidence that the serum of an animal which has been inoculated with typhoid bacteria contains not one, but several agglutinins of different degrees of activity. In view of these facts, any argument as to the nature of agglutination based on the applicability of the adsorption formula appears to be open to criticism.

CHAPTER XII

CHEMICAL EQUILIBRIUM AND THE LAW OF MASS ACTION

Reversible Reactions.—The chemical reactions employed for the purposes of analytical chemistry may be described as 'complete' reactions, for they are such that they proceed until one or other of the reacting compounds has entirely disappeared. Suppose, looking at things from the standpoint of the analytical chemist, we take the change on which the ordinary method of detecting and estimating silver or chloride in solution depends. This change is represented by the equation $AgNO_3 + NaCl = AgCl + NaNO_3$, and it is well known that the reaction proceeds until either the silver nitrate or the sodium chloride is completely removed; short of that there is no halt in the reaction. Similarly, the reaction between hydrochloric acid and sodium hydroxide in aqueous solution proceeds until either one or the other disappears; they cannot exist together in the same solution. It is noteworthy that such complete reactions are, indeed must be, non-reversible. It is impossible, for instance, to regenerate silver nitrate and sodium chloride from a suspension of silver chloride in sodium nitrate solution, certainly not to an extent which can be detected by ordinary analytical methods.

There are, however, numerous reactions which may be described as 'incomplete': they do not proceed until one or other of the reacting substances has completely disappeared. The reaction stops short at an equilibrium

point at which the products of the change, and the original substances as well, are all represented in the reaction mixture. Such reactions, too, are 'reversible'; that is, the substances represented on the right-hand side of the equation will, if brought together, react to produce the substances represented on the left-hand side of the equation; further, an equilibrium point is reached which, provided that equivalent quantities of the reagents have been taken in both cases, is the same point as is attained by starting with the substances on the left side of the equation.

An illustration of such reversibility is furnished by the reaction between hydrogen and iodine. If a small quantity of iodine is introduced into a glass bulb, and the bulb is then filled with hydrogen, sealed off and exposed to a temperature of, say, 440°, the two elements begin to combine. After an hour or two, however, the reaction stops, and if the bulb is cooled and opened, it is found to contain hydrogen iodide, hydrogen, and iodine. If, on the other hand, the bulb were filled with pure hydrogen iodide and kept at 440° until no further change took place, it would be found that the bulb, when cooled and opened, contained hydrogen iodide, hydrogen, and iodine, as in the other case. The reaction therefore is reversible, and this fact may be indicated by substituting oppositely directed arrows for the usual sign of equality in the equation representing the change, thus: $H_2+I_2 \rightleftarrows 2HI$.

Another standard case of reversibility is the reaction between ethyl alcohol and acetic acid. When 1 gram-mol. of ethyl alcohol is mixed with 1 gram-mol. of acetic acid, a reaction takes place resulting in the formation of ethyl acetate and water; the reaction, however, is incomplete, and stops at an equilibrium point at which the reaction mixture contains $\frac{1}{3}$ gram-

mol. alcohol, $\frac{1}{3}$ gram-mol. acid, $\frac{2}{3}$ gram-mol. ethyl acetate, and $\frac{2}{3}$ gram-mol. water. If, on the other hand, 1 gram-mol. of ethyl acetate is mixed with 1 gram-mol. of water, a reaction sets in resulting in the formation of ethyl alcohol and acetic acid. This change likewise stops at an equilibrium point at which the composition of the reaction mixture is the same as that already stated. Since the reaction is thus reversible, it may be written $C_2H_5OH + CH_3.COOH \rightleftarrows CH_3.COOC_2H_5 + H_2O$.

Law of Mass Action Applied to Reversible Reactions.—The law of mass action states that the velocity of a chemical reaction is proportional to the molecular concentration of each of the reacting substances. The line of proof of this law may be traced by considering a reaction which takes place between two gases A and B, and by looking at matters from the molecular-kinetic standpoint. In such a case the reaction can take place only in so far as the molecules of A come into contact with the molecules of B. The velocity of the reaction, therefore—that is, the rate at which A and B disappear—will be proportional to the frequency of the collisions between a molecule of A and a molecule of B, even although only a certain proportion of the collisions is followed by chemical interaction. But, on kinetic grounds, the frequency of the collisions between a molecule of A and a molecule of B is proportional to the product of their molecular concentrations, hence it follows that the velocity of reaction between A and B is proportional to the product of their molecular concentrations (or their 'active masses,' as it is sometimes put). A similar line of argument may be followed in the case where A and B are dissolved substances.

Suppose, now, that we are dealing with a reversible reaction, represented by $A + B \rightleftarrows C + D$, and suppose that the four substances are mixed together so that in the

mixture their (molecular) concentrations are a_0, b_0, c_0, and d_0 respectively. If these are not the proportions corresponding to the equilibrium point a reaction will take place, from left to right or *vice versâ* according to the circumstances, and will continue until equilibrium is established. The velocity of the reaction may obviously be resolved into two component opposing velocities, firstly V_1, the rate at which A and B are reacting to form C and D, and secondly V_2, the rate at which C and D are reacting to form A and B. The difference between V_1 and V_2 is the observed velocity of the reaction. Now, at the moment of mixing, according to the law of mass action, $V_1 = k_1 a_0 b_0$ and $V_2 = k_2 c_0 d_0$, where k_1 and k_2 are proportionality factors, so that the observed velocity immediately after mixing is $V_1 - V_2 = k_1 a_0 b_0 - k_2 c_0 d_0$. After the reaction has proceeded for some time, and has consequently approached the equilibrium position, the values of the concentrations will be different, say, a, b, c, and d. The velocity of the reaction will therefore now be $k_1 ab - k_2 cd$. If the reaction has proceeded long enough to reach the equilibrium point, at which we may suppose the concentrations are a_e, b_e, c_e, d_e, then the velocity of the reaction is zero, and $k_1 a_e b_e = k_2 c_e d_e$. This may be written $\frac{k_1}{k_2} = \frac{c_e d_e}{a_e b_e}$ or $K = \frac{c_e d_e}{a_e b_e}$, where K is a constant independent of the concentrations of the reacting substances, depending only on the nature of the reaction and the temperature. K is known as the equilibrium constant, and the significance of the equilibrium formula may be stated in the following terms: For any reversible reaction at a given temperature, the product of the equilibrium concentrations of the substances on the right-hand side of the equation stands in a constant ratio to the corresponding product for the substances on the left-hand side.

The extent to which this application of the law of

mass action to reversible reactions is verified by experiment is best appreciated by a more detailed consideration of the reaction between ethyl alcohol and acetic acid: $C_2H_5OH + CH_3COOH \rightleftarrows CH_3.COOC_2H_5 + H_2O$. It has been stated already that the equilibrium system reached after mixing 1 gram-mol. of alcohol and 1 gram-mol. of acid contains $\frac{1}{3}$ gram-mol. of alcohol, $\frac{1}{3}$ gram-mol. of acid, $\frac{2}{3}$ gram-mol. of ester, and $\frac{2}{3}$ gram-mol. of water. If v is the volume of the system at the point of equilibrium, then the molecular concentrations of the four substances are $\frac{\frac{1}{3}}{v}$, $\frac{\frac{1}{3}}{v}$, $\frac{\frac{2}{3}}{v}$, and $\frac{\frac{2}{3}}{v}$ respectively. Hence $K = \frac{c_c d_e}{a_a b_e} = \frac{\frac{2}{3}.\frac{2}{3}}{\frac{1}{3}.\frac{1}{3}} = 4$.

If, now, the law of mass action is strictly applicable to this reversible reaction, then we ought to find the same value of K even when the initial proportions of alcohol and acid are quite different. Suppose, for instance, that 1 gram-molecule of acetic acid is mixed with m gram-molecules of alcohol, and that the reaction is allowed to proceed to the equilibrium point. If x is the fraction of a gram-molecule of ester which is present in the equilibrium mixture, then the corresponding quantities of acid, alcohol, and water are $1-x$, $m-x$, and x respectively; further, if v is the volume of the equilibrium mixture, the concentrations of acid, alcohol, ester, and water are $\frac{1-x}{v}$, $\frac{m-x}{v}$, $\frac{x}{v}$, and $\frac{x}{v}$ respectively. Applying the equilibrium formula we obtain $K = \frac{\frac{x}{v}.\frac{x}{v}}{\frac{1-x}{v}.\frac{m-x}{v}} = \frac{x^2}{(1-x)(m-x)}$. The value of x is obtained by determining the amount of free acetic acid in the equilibrium mixture; this is permissible, since the velocity of the reaction becomes appreciable only at high temperatures; at the ordinary temperature the free acid may be removed by neutralisation without the back reaction setting in to any appreciable extent. From the

known values of m and x it is then possible to calculate K, and if the law of mass action is valid, the value so calculated ought to be the same as that obtained with equivalent quantities of the reacting substances. There is, however, another way in which the applicability of the law may be tested, namely, by taking $K=4$, the figure already recorded, and calculating for each value of m what the value of x ought to be on the basis of the equilibrium formula. The comparison of the values of x so calculated and those directly observed is made in the following table:—

m.	x (found).	x (calc.).
0·08	0·078	0·078
0·28	0·226	0·232
0·50	0·414	0·423
0·67	0·519	0·528
1·5	0·819	0·785
2·24	0·876	0·864
8·0	0·966	0·945

The excellent agreement of the figures in the second and third columns demonstrates the applicability of the law of mass action to the reversible reaction between ethyl alcohol and acetic acid. A glance at the table further shows that, although by treating acetic acid with the equivalent quantity of alcohol it is possible to convert only 66·6 per cent. of the acid into ester, yet by using a large excess of alcohol practically the whole of the acid can be converted into ester. Similarly, the first line of figures in the table shows that when the acid is in large excess, practically the whole of the alcohol is converted into ester.

The same features characterise every reversible reaction. For each such there is a formula with a characteristic constant which defines the relationship between the reacting substances at the point of equilibrium.

Application of the Law of Mass Action to Electrolytes. Ostwald's Dilution Law.—On the ground of evidence submitted in earlier chapters the view has been adopted that an electrolyte AB, dissolved in water, is to a greater or less extent dissociated into its ions A' and B', and that the degree of dissociation increases with dilution of the solution. The equilibrium, therefore, between an undissociated electrolyte and its ions may be shifted in one direction or the other by simply diluting or concentrating the solution. The process of dissociation is, in fact, a reversible action, and may be represented as $AB \rightleftarrows A' + B'$. As Ostwald pointed out, the law of mass action ought to be applicable in such a case. Suppose that in volume V of the solution there is altogether 1 gram-equivalent of electrolyte, and that the degree of dissociation is α; then the quantity of the undissociated electrolyte, stated as fraction of a gram-equivalent, is $1-\alpha$, and the quantity of each ion, similarly expressed, is α; the concentrations are $\frac{1-\alpha}{V}$ and $\frac{\alpha}{V}$ respectively. The equilibrium formula in this case is $K = \frac{\frac{\alpha}{V} \cdot \frac{\alpha}{V}}{\frac{1-\alpha}{V}} = \frac{\alpha^2}{(1-\alpha)V}$, which means that, according to the law of mass action, the degree of dissociation must so vary with the dilution that $\frac{\alpha^2}{(1-\alpha)V}$ is a constant for the particular electrolyte chosen. The equation $K = \frac{\alpha^2}{(1-\alpha)V}$ is the algebraic expression of what is known as Ostwald's Dilution Law.

As an example of a case where the validity of Ostwald's Dilution Law is amply verified, we may take acetic acid. The value of α, which is required for the calculation of K, is most easily ascertained by determining the conductivity as described in Chapter VII. The values of α so deduced for a number of acetic acid

solutions containing 1 gram-equivalent of the acid in V litres are recorded in the following table; the corresponding values of K are entered in the last column:—

V.	a.	$K \times 10^5$.
0·994	0·004	1·62
2·02	0·00614	1·88
15·9	0·0166	1·76
18·1	0·0178	1·78
1500	0·147	1·69
3010	0·205	1·76

The values of K are satisfactorily constant, for it must be observed that, owing to the very character of the equilibrium formula, the value of K is very sensitive to experimental error. Thus, for example, if a for the first solution had been found to be 0·0041 instead of 0·0040, the value of $K \times 10^5$ would have been 1·70 instead of 1·62. The figure usually taken as the value of K for acetic acid—the 'dissociation constant'—is $1{\cdot}8 \times 10^{-5}$ at 25°. The dissociation constant of all weak (that is, slightly dissociated) acids and weak bases may be determined in a similar fashion, but it is remarkable that for strong acids, such as hydrochloric acid, for strong bases, such as sodium hydroxide, and for neutral salts, such as potassium nitrate, the value of $\frac{a^2}{(1-a)V}$ varies regularly with dilution. The fact that the behaviour of strong acids, strong bases, and neutral salts is not in harmony with Ostwald's dilution law has not yet been satisfactorily explained, and indicates one direction in which the electrolytic dissociation theory requires to be supplemented.

It is instructive to compare the values of K obtained for different organic acids. A few typical cases are given below. It is noteworthy how the introduction of chlorine into the acetic acid molecule increases the

Acid.	K.
Acetic	0·000018
Monochloracetic . . .	0·00155
Dichloracetic	0·0514
Formic	0·000214
Benzoic	0·00006
Salicylic	0·00102

value of the dissociation constant; the much greater value of K for salicylic acid as compared with benzoic acid is also interesting.

The Strength of Acids.—The occurrence of such notable changes in the value of K from one acid to another raises the question of the significance of the dissociation constant. As already stated, $K=\frac{a^2}{(1-a)V}$, so that if we are considering two acids which are feebly dissociated, for which therefore $1-a$ is practically 1, and if we compare the two acids at the same concentration, we have $K_1=\frac{a_1^2}{V}$ and $K_2=\frac{a_2^2}{V}$. From this it appears that the value of K for an acid is an expression of its inherent ability to dissociate into its ions. If, now, we remember that all acids are alike in splitting off the hydrogen ion, and that with this ion are associated all those properties which are characteristic of acids as a class, it follows that the dissociation constant of an acid is a measure of its ability to exhibit those characteristic properties. The value of K, in other words, is a measure of the strength of the acid. If this is so, then the order of the acids arranged according to their values of K ought also to be the order of their strength deduced on other grounds. This turns out to be the case, as shown by the investigations of Ostwald and others. The strength of two acids may be compared by allowing them to compete for an insufficient quantity of a base, and then to determine, from the volume

changes which occur, what proportion of the base has been appropriated by each acid. Or the effect of each acid in promoting the inversion of cane sugar may be determined (see pp. 167, 277); the rate of this change is approximately proportional to the concentration of the hydrogen ions present, and may be employed to compare the degrees of dissociation of two acids in equivalent concentration.

That a base is shared by two acids in proportions depending on their relative strength is an important fact, which is demonstrated by the following experiment. A dilute solution of the sodium salt of p-nitrophenol is prepared, and equal portions of the solution are put in three test glasses. The solution, it should be noted, has an intense yellow colour, whereas the p-nitrophenol itself, when dissolved in distilled water, gives a very pale yellow solution. Accordingly, when standard hydrochloric acid, say $\frac{N}{2}$, is gradually added to one of the portions, a point is reached at which the colour is almost completely discharged; this is the point at which enough hydrochloric acid (say x cub. cm.) has been added to turn out practically all the p-nitrophenol from its combination with the soda; in competition with such a weak acid as p-nitrophenol, hydrochloric acid is able to appropriate practically the whole of the base. If, however, x cub. cm. of $\frac{N}{2}$ monochloracetic acid are added to the second portion of the salt solution, the colour is not discharged; it remains distinctly yellow, showing that in competition with monochloracetic acid, which is much weaker than hydrochloric acid, p-nitrophenol is able to retain some of the soda. If to the third portion of the sodium salt solution x cub. cm. $\frac{N}{2}$ acetic acid are added, the colour of the

mixture is markedly more intense than in the second case, for now, since acetic acid is comparatively weak, the p-nitrophenol retains an appreciable fraction of the available soda.

If the relative strength of two acids has been determined by studying their influence in equivalent concentration on the rate of inversion of cane sugar, the figure obtained is valid only for the concentration in question. That this must be so is evident if we take the particular case of hydrochloric and acetic acids. From conductivity data it follows that the dissociation of acetic acid, although small, increases much more rapidly with dilution than that of hydrochloric acid, so that the disparity between the two acids in regard to their content of hydrogen ions diminishes with increasing dilution. Since the rate of inversion of cane sugar is approximately proportional to the concentration of hydrogen ions, it is only to be expected that the strength of $\frac{N}{100}$ acetic acid (measured in terms of the effect of $\frac{N}{100}$ hydrochloric acid on the rate of sugar inversion) is greater than the strength of $\frac{N}{1}$ acetic acid (measured in terms of the corresponding effect of $\frac{N}{1}$ hydrochloric acid). This point is illustrated by the following figures for the relative influence of hydrochloric and monochloracetic acids on the rate of inversion of sucrose; the figure for hydrochloric acid is in each case taken as 100:—

	$\frac{N}{1}$ Acid.	$\frac{N}{25}$ Acid.
HCl	100	100
$CH_2Cl.COOH$	5	14

If this argument is followed out, it is obvious that at infinite dilution all acids would be equally strong.

Strength of an Acid as Affected by its Salts.—The equilibrium between the undissociated molecule of a weak acid and its ions is, as we have seen, a reversible one, and it is possible therefore to shift the equilibrium in either direction according to the conditions. The evidence submitted in connection with the reversible reaction between ethyl alcohol and acetic acid showed that the greater the concentration of alcohol in the equilibrium mixture the smaller was the corresponding concentration of acetic acid. This is an illustration of a general principle, which, if applied to the equilibrium between, say, acetic acid and its ions, $CH_3.COOH \rightleftharpoons CH_3.COO' + H^{\cdot}$, shows that by increasing the concentration of acetate ions the concentration of hydrogen ions would be diminished. It is easy to increase the concentration of the acetate ions by adding sodium acetate, solutions of which are shown by conductivity data to be highly dissociated. We should therefore expect that an acetic acid solution to which sodium acetate has been added would contain fewer hydrogen ions than an equally concentrated solution of acetic acid to which no sodium acetate has been added; the acid effect would be weakened by the presence of a neutral salt of the acid. This conclusion is strikingly verified by the figures given in the following table.[1] V represents the rate of inversion of sucrose under the influence of $\frac{N}{4}CH_3COOH$ in presence of gradually increasing quantities of sodium acetate: the actual method by which V is determined will be described later, and for the present it may simply be taken as a measure of the concentration of hydrogen ions in the acetic acid solution. The numbers in the column

[1] Arrhenius, *Zeit. physikal. Chem.*, 1890, 5, 1.

headed V obs. show that, in accordance with the argument outlined above, the concentration of hydrogen ions in $\frac{N}{4}CH_3COOH$ diminishes steadily as the concentration of sodium acetate in the same acetic acid solution is increased. More than that, it is possible, on the basis of the equilibrium formula, to calculate the concentration of hydrogen ions in each solution, and therefrom to calculate the velocity of inversion under the influence of that solution; the values so obtained are tabulated under V calc.

Inverting Solution.	V obs.	V calc.
$\frac{N}{4}CH_3COOH$	0·75	...
" $+\frac{N}{80}CH_3COONa$	0·122	0·129
" $+\frac{N}{40}$ "	0·070	0·070
" $+\frac{N}{20}$ "	0·040	0·038
" $+\frac{N}{8}$ "	0·019	0·017
" $+\frac{N}{4}$ "	0·0105	0·0100

The agreement between the observed and calculated values is striking evidence in favour of the electrolytic dissociation theory, which is involved in the calculation.

It must, however, be pointed out that such a calculation cannot be successfully made for the influence of neutral salts on the inverting efficiency of strong acids. In one sense this is not strange, since, as already indicated, Ostwald's dilution law is not valid for these. But the influence of, say, sodium chloride on the activity of hydrochloric acid is not even qualitatively in agreement with what we should expect on the basis of the electrolytic dissociation equilibrium. The rate at which sucrose is inverted by hydrochloric acid is *increased* by the addition

of a neutral chloride, an effect that is generally referred to as 'neutral salt action.' The magnitude of the effect is considerable, for Arrhenius has shown that the rate of inversion of a 10 per cent. sucrose solution by 0·05NHCl is increased by about 25 per cent. when the solution is also 0·4N in relation to sodium chloride. Other neutral chlorides exert a like accelerating influence. Neutral salt action has not yet been satisfactorily explained, although numerous attempts have been made;[1] Arrhenius, for instance, suggests that neutral salts increase the osmotic pressure of the sucrose, while Caldwell[2] regards neutral salt action as a concentrating effect brought about by the hydration of the salt. In any case, some factor is involved in neutral salt action of which the electrolytic dissociation theory, in its original form at least, takes no cognisance.

Reactions between Ions.—The influence of sodium acetate or any other neutral acetate in repressing the dissociation of acetic acid is one example of the ionic reactions which occur when solutions of electrolytes are mixed, and of which only indirect evidence can be obtained. If a little concentrated sodium acetate solution is added to a solution of hydrochloric acid nothing obvious happens, but indirect evidence can be obtained showing that a reaction does indeed take place, which results in the almost complete removal of the hydrogen ions from the solution. If, for instance, sulphuretted hydrogen water is added to a solution of ferrous sulphate which has been acidified with hydrochloric acid, no precipitate is produced; on the further addition, however, of a little concentrated sodium acetate solution, a black precipitate of ferrous sulphide is thrown down immediately. The

[1] See Senter, *Journ. Chem. Soc.*, 1907, 91, 462.

[2] *Proc. Roy. Soc.*, A, 1906, 78, 272.

sodium acetate impairs the acid effect of the hydrochloric acid, and a consideration of the phenomenon from the point of view of electrolytic dissociation leads to an intelligible explanation. When hydrochloric acid and sodium acetate, both highly dissociated electrolytes, are mixed in aqueous solution, opportunity is given for the formation of two other electrolytes, namely, sodium chloride and acetic acid, the first of which is highly, the second feebly, dissociated. As we have seen already, the dissociation constant of acetic acid is low, which means that acetate ions and hydrogen ions can exist alongside each other only to a certain small extent, defined by the said dissociation constant. Hence when hydrochloric acid and sodium acetate are mixed, the acetate ions and hydrogen ions unite almost completely to form undissociated acetic acid, and the concentration of free hydrogen ions is still further diminished if excess of sodium acetate is added to the hydrochloric acid. If we make the assumption, which is not very far from the truth, that hydrochloric acid, sodium acetate, and sodium chloride are almost completely dissociated, while acetic acid is dissociated to a negligible extent, the main course of the ionic reaction in question is expressed by the equation

$$H^{\cdot} + Cl' + Na^{\cdot} + CH_3.COO' = CH_3.COOH + Cl' + Na^{\cdot}.$$

The addition of sodium acetate to hydrochloric acid thus effects a removal of the hydrogen ions, and so amounts practically to a neutralisation of the great bulk of the hydrochloric acid. The sodium salt of any weak acid would do quite as well as sodium acetate; if, for instance, a strong solution of borax is added to a solution containing ferrous sulphate, sulphuretted hydrogen water, and a little free hydrochloric acid, a black precipitate

is produced immediately. The explanation of this effect is the same as that given in the case of sodium acetate.

Another ionic reaction which occurs on mixing two electrolytes which have no common ion is the union of the hydrogen and hydroxyl ions. These ions cannot exist alongside each other except in the minutest quantities (see p. 170), so that the process of neutralisation of hydrochloric acid (or any other strong acid) by sodium hydroxide (or any other strong base) may be represented by the equation: $H^{\cdot}+Cl'+Na^{\cdot}+OH'=H_2O+Cl'+Na^{\cdot}$. The similarity between this neutralisation and the result of adding sodium acetate to hydrochloric acid is apparent.

Just as the equilibrium between acetate ions, hydrogen ions, and undissociated acetic acid is defined by the dissociation constant for acetic acid, so the extent to which hydrogen and hydroxyl ions can exist alongside each other in water is similarly fixed. If we apply the law of mass action to the equilibrium $H_2O \rightleftarrows H^{\cdot}+OH'$ we obtain $K=\frac{C_H.C_{OH}}{C_{H_2O}}$, where C_H, C_{OH}, and C_{H_2O} are the concentrations of the hydrogen ion, the hydroxyl ion, and water respectively. Since the concentrations of the ions are extremely small, that of the water may be taken as independent of their variations, so that $C_H.C_{OH}=k$, another constant. This means that the product of the concentrations of the hydrogen and hydroxyl ions in any aqueous solution must be a constant. Several lines of evidence lead to the figure $1{\cdot}2\times10^{-14}$ being taken as the value of k at 25° (compare pp. 172 and 317).

If water contains both hydrogen and hydroxyl ions in equivalent quantities, it may be regarded as a very weak acid or as a very weak base, so that when a neutral salt AB is dissolved in the water—a salt which

is largely dissociated into its ions A′ and B• — there is the possibility of ionic reactions taking place which result in the formation of two new undissociated compounds, HA and BOH. The extent to which this takes place will depend on the strengths of the acid and the base. If they are both very strong, as would be the case if AB stood for NaCl, the quantities of HA and BOH formed will be very small, and approximately equal quantities of hydrogen and hydroxyl ions will be removed for the purpose. This removal of hydrogen and hydroxyl ions is made good by the dissociation of a small quantity of water, in order to maintain the condition $C_H.C_{OH} = 1{\cdot}2 \times 10^{-14}$. Suppose, however, that HA is a weak acid, while BOH is a strong base—as would be the case if AB stood for borax—then from the ions A′, B•, H•, and OH′ more undissociated HA will be formed than undissociated BOH, and there will no longer be equivalent quantities of hydrogen and hydroxyl ions. Although to maintain the condition $C_H.C_{OH} = 1{\cdot}2 \times 10^{-14}$ a little water will dissociate, this cannot get rid of the excess of hydroxyl ions; the solution will therefore have an alkaline reaction. In harmony with this it is found that borax, however carefully it is purified, always gives an alkaline reaction when dissolved in water. Other salts which behave in a similar way, since they are derived from a strong base and a weak acid, are potassium cyanide and sodium carbonate.

If the salt AB, on the other hand, is such that HA is a strong acid, while BOH is a weak base, the solution of the salt must have an acid reaction. The argument which leads to this conclusion is parallel to that already given. The case where a salt, however carefully purified, gives an acid reaction when dissolved in water is illustrated by aniline hydrochloride.

The phenomenon of a neutral salt reacting with

water so as to give either an acid or an alkaline reaction is known as 'hydrolytic dissociation.' If we wish to determine the extent of hydrolytic dissociation in any given case, we observe the influence of the salt on the rate of inversion of sucrose or on the rate of saponification of ethyl acetate (see p. 283). The first of these methods is employed when the salt in question is derived from a strong acid and a weak base, the second when it is derived from a strong base and a weak acid. In this way it has been found that in $\frac{N}{10}$ solution at 25° sodium carbonate is hydrolytically dissociated to the extent of 3·17 per cent., potassium cyanide 1·12 per cent., borax 0·05 per cent., aniline hydrochloride 1·5 per cent.

Amphoteric Electrolytes.—Water has been described above as an electrolyte which yields at the same time hydrogen and hydroxyl ions. There is an interesting class of substances which in this respect resemble water, and are, further, of considerable importance in connection with the behaviour of proteins. The class referred to is that of the amino-carboxylic acids, substances which contain at least one NH_2-group and one COOH-group, and are therefore capable of acting as bases or as acids according to circumstances; in view of this double character they are termed 'amphoteric' electrolytes. Such an electrolyte will have two dissociation constants, one corresponding to its acid function, the other to its basic function. It is further capable of forming two series of salts, one series by combining with acids, the other series by combining with bases.

Just as ammonia, when dissolved in water, exists, to some extent at least, in the form of the compound NH_4OH, so it is supposed that glycine, which may be taken as an example of an amino-acid, forms in water

the compound $\begin{matrix}CH_2.NH_3.OH\\|\\COOH\end{matrix}$. The ions produced by the dissociation of this compound are

$$OH', H', \begin{matrix}CH_2.NH_3\cdot\\|\\COOH\end{matrix}, \begin{matrix}CH_2.NH_3.OH,\\|\\COO'\end{matrix} \text{ and } \begin{matrix}CH_2.NH_3\cdot\\|\\COO'\end{matrix};$$

the last mentioned results from the simultaneous splitting off of hydrogen and hydroxyl ions, and corresponds really to an intramolecular salt. The concentrations of all these ions are exceedingly small in an ordinary aqueous solution of glycine; the acid and basic groups present in the molecule are antagonistic, and the result is that glycine, whether regarded as acid or base, is very weak. A similar statement applies to all amino-carboxylic acids.

This being so, it is clear that the salts of such an amphoteric electrolyte, both with a strong acid and a strong base, will be liable to hydrolytic dissociation. Suppose that HROH represents the formula of an amino-carboxylic acid, and that the sodium salt NaROH and the chloride HRCl have been prepared; this is possible, since the amino-carboxylic acid acts as an acid towards sodium hydroxide and as a base towards hydrochloric acid. The salt NaROH, being derived from a strong base and a weak acid, will be hydrolytically dissociated in aqueous solution, an effect which may be regarded as brought about by an interaction between water and the negative ion of the salt, thus:

$$ROH' + H_2O \rightleftarrows HROH + OH'.$$

The hydrolytic dissociation results, therefore, in the negative ion ROH′ being replaced to some extent by the OH′ ion. The ionic conductivity of the hydroxyl ion is much greater than that of any other anion, hence one result of the hydrolytic dissociation of NaROH is that the conductivity of the solution is exceptionally high. A determination of the conductivity may in fact be used

to calculate the extent of the hydrolytic dissociation. This may be ascertained also by studying the influence of the salt NaROH on the rate of saponification of ethyl acetate, which, as already stated, is a measure of the hydroxyl ion concentration. The extent of hydrolysis of the salt NaROH having been ascertained by one or other of these methods, it is possible to calculate the acidic dissociation constant k_a of HROH; the way in which this is done cannot be discussed here.

Similarly, by determining the conductivity of the salt HRCl, or by studying its influence on the rate of inversion of sucrose, the extent of hydrolytic dissociation in this case can be ascertained, and the basic dissociation constant k_b deduced therefrom. For amino-carboxylic acids it is found generally that k_a is greater than k_b; that is, the acidic character of these compounds is more strongly developed than their basic character. The following figures may be quoted in support of this statement:[1]—

	$ka(25°)$	k_b (25°).
Glycine	$1{\cdot}8 \times 10^{-10}$	$2{\cdot}7 \times 10^{-12}$
Sarcosine	$1{\cdot}2 \times 10^{-10}$	$1{\cdot}7 \times 10^{-12}$
Alanine	$1{\cdot}9 \times 10^{-10}$	$5{\cdot}1 \times 10^{-12}$
Leucine	$1{\cdot}8 \times 10^{-10}$	$2{\cdot}3 \times 10^{-12}$
β-Asparagine . . .	$1{\cdot}35 \times 10^{-9}$	$1{\cdot}53 \times 10^{-12}$

The values for k_a recorded in the table are about the same as k_a for phenol, so that the acidic character of all these amino-carboxylic compounds is exceedingly feeble. Their basic character is still less marked; the values of k_b in the table are roughly about one-hundredth of the corresponding figure for aniline.

In the case of a sparingly soluble amphoteric electrolyte, its peculiar character is clearly indicated by the influence of acids and alkalis on its solubility. A sparingly soluble base, aniline for instance, is more soluble

[1] See Lundén, *Zeit. physikal. Chem.*, 1906, 54, 561.

in dilute hydrochloric acid, but not more soluble in dilute sodium hydroxide, than it is in water. A sparingly soluble acid, salicylic acid for instance, is conversely more soluble in dilute sodium hydroxide, but not more soluble in dilute hydrochloric acid, than it is in water. We should expect therefore that the solubility of a sparingly soluble amphoteric electrolyte, which functions both as acid and as base, would be increased by adding either acid or alkali. This turns out to be the case, as has been shown, for instance, in the case of theobromine. Paul found[1] that one part of theobromine required for its solution 3282 parts of water at 18°, 2125 parts of $\frac{N}{4}$HCl, or 22·93 parts of $\frac{N}{4}$NaOH. The solubility of theobromine in acid is little greater than its solubility in water, which means that the hydrochloride is hydrolytically dissociated to a very large extent, and that the basic character of theobromine is therefore feebly developed. As is evident from the comparative solubility in sodium hydroxide, the acidic character of theobromine is well marked; k_a in this case is $1{\cdot}33 \times 10^{-8}$.

There is every reason to believe that the polypeptide group forms an essential part of the protein molecule,[2] and as polypeptides are built up by the condensation of amino-carboxylic acids, there is good ground for regarding the proteins as amphoteric electrolytes. In many respects their behaviour is in harmony with this conception of their character. There is, for instance, the observation, due originally to Hardy and confirmed by Pauli,[3] that neutral protein acquires electro-positive characteristics on the addition of acids, as shown by its

[1] *Arch. Pharm.*, 1901, 239, 48.

[2] See Schryver, *The General Characters of the Proteins.*

[3] *Hofmeister's Beitr.*, 1906, 7, 531.

migration towards the cathode in an electric field, while it acquires electro-negative characteristics on the addition of alkali.

Further support for the view that protein is an amphoteric substance is furnished by the work of Bugarszky and Liebermann,[1] who studied the effect of adding egg albumin to 0·05N solutions of hydrochloric acid, sodium hydroxide, and sodium chloride. The effect was measured by determining the freezing points of the electrolyte solutions (1) without any albumin, (2) after the addition of various quantities of albumin. Some of the results obtained are incorporated in the following table, the first column giving the weight (g) of albumin added to 100 cub. cm. of the electrolyte solution, while the three succeeding columns give the observed depressions (Δ) of the freezing point:—

g.	Δ for 0·05N HCl.	Δ for 0·05N NaOH.	Δ for 0·05N NaCl.
0	0·186°	0·181°	0·183°
0·8	0·172°	0·162°	0·191°
1·6	0·146°	0·151°	0·194°
3·2	0·107°	0·116°	0·199°
6·4	0·087°	0·097°	0·203°

The depression of the freezing point in the case of sodium chloride is increased by the addition of albumin, and the amount of the increase is practically equal to the depression which the albumin produces by itself; thus a solution containing 6·4 grams of egg albumin in 100 grams of water had a freezing point 0·022° below that of water. The effect of egg albumin on the freezing points of 0·05N hydrochloric acid and sodium hydroxide is obviously quite a different phenomenon. The depression of the freezing point produced by the given quantity of acid or alkali diminishes markedly as the quantity of added albumin increases. This shows clearly

[1] *Pflüger's Arch.*, 1898, 72, 51.

that the number of molecules originally present in the acid or alkali solution has decreased, and this must be due to the ability of both acid and alkali to form complex molecules with the albumin.

Dissociation Equilibrium in a Saturated Solution of an Electrolyte.—The systems to which we have hitherto applied the law of mass action have been homogeneous—mainly solutions of electrolytes. It will be interesting now to see in what way the law works out when applied to a non-homogeneous system, consisting, say, of a saturated solution of an electrolyte in contact with excess of the solid substance. Suppose we take the case of benzoic acid, an electrolyte to which Ostwald's dilution law is applicable. In a saturated solution of this acid we have equilibrium between the undissociated molecules and the ions, as represented by the following:

$$C_6H_5.COOH \rightleftarrows C_6H_5.COO' + H'.$$

There is however this peculiarity, that we cannot alter the concentration of the undissociated molecules so long as the temperature remains constant, for by supposition the solution is saturated with benzoic acid, and is in contact with solid benzoic acid. Thus if anything happened to increase the concentration of the undissociated molecules, this would simply lead to an equivalent removal of acid from solution. If anything happened to diminish the concentration of the undissociated molecules, fresh acid would dissolve until the said concentration was brought up to its saturation value. That is to say, the concentration or active mass of the undissociated benzoic acid molecules in the above dissociation equilibrium is constant, so long as there is excess of benzoic acid present and the temperature remains constant. The application of the law of

mass action to the equilibrium between benzoic acid and its ions leads to $K=\frac{c_1c_2}{c}$, where c_1 and c_2 are the concentrations of the ions, and c is that of the undissociated molecules. As has just been explained, c is constant under the specified conditions, so that $c_1c_2=\text{const.}$ Naturally, so long as the solution contains nothing but benzoic acid, $c_1=c_2$, but if the equilibrium between benzoic acid and its ions is displaced by the introduction of other electrolytes, c_1 will be different from c_2; even then, however, the law of mass action requires the condition $c_1c_2=\text{const.}$ to be fulfilled. This means that for any solution which is kept saturated with benzoic acid at a given temperature the product of the concentrations of the ions remains constant, however their individual values may vary. This product of the ionic concentrations in a saturated solution is generally known as the 'solubility product.'

In considering the conditions which define the equilibrium in a saturated solution of a sparingly soluble electrolyte we have taken a special case. This case, however, serves to bring out two general principles involved in a non-homogeneous equilibrium; these may be stated as follows: (1) The active mass or concentration of any solid concerned in a non-homogeneous equilibrium is constant for a given temperature; (2) for any dissociation equilibrium in a saturated solution the product of the concentrations of the dissociated parts is a constant for a given temperature.[1]

One or two consequences of the application of these principles to saturated solutions of electrolytes are worth noting. If the product of the ionic concentrations c_1c_2 is to remain constant, anything which leads to an increase of c_2 must mean a diminution of c_1. Now, as pointed out in the previous part of this chapter,

[1] See, however, Kendall, *Proc. Roy. Soc.*, A, 1911, 85, 200.

it is possible to increase the concentration of one of the ions involved in an electrolytic dissociation equilibrium by adding another electrolyte with a common ion. The result of this is to repress the dissociation of the first electrolyte, that is, to increase the concentration of the undissociated molecules. If, now, the solution is already saturated with this first electrolyte, it cannot contain any more of the undissociated molecules; the consequence is that some of the electrolyte separates out in solid form. The law of mass action, then, applied to the dissociation equilibrium in the saturated solution of an electrolyte AB, leads us to expect that the addition of another electrolyte which yields either A' or B' as one of its ions, will throw some of the compound AB out of solution; in other words, will lower the solubility of AB. This conclusion is amply verified by experiment. If to a saturated solution of barium nitrate we add a little concentrated nitric acid, solid barium nitrate is precipitated; the addition of a little concentrated solution of either silver nitrate or sodium acetate to a saturated solution of silver acetate throws down some of the latter salt. The following figures give a more definite shape to the results of experiment:[1] they represent the extent to which the solubility of thallous chloride at 25° is affected by the presence of an electrolyte with a common ion, namely, either thallous nitrate or hydrochloric acid; all figures are given in gram-mols. per litre:—

	Solubility of Thallous Chloride.	
Concentration of the added Electrolyte.	In Presence of $TlNO_3$.	In Presence of HCl.
0·0	0·0161	0·0161
0·0283	0·0083	0·00836
0·0560	0·00571	0·00565
0·1468	0·00332	0·00316

The diminution in the solubility brought about by

[1] See Noyes, *Zeit. physikal. Chem.*, 1890, 6, 249.

increasing quantities of an electrolyte with a common ion is very marked, and a comparison of the figures in the last two columns shows that the effect is pretty much the same whether the common ion is anion or cation, provided the added electrolytes are dissociated to about the same extent. On these lines also we get an intelligible explanation of the practice, common in analytical operations, of adding a slight excess of a precipitating reagent; any slight solubility which the precipitate may have is thereby reduced.

What, it may be asked, would be the result of adding to a saturated solution of an electrolyte another electrolyte which has no ion common with the first? The principles already laid down enable us to deal with this case. For the addition of an electrolyte with no common ion makes possible the formation of two new undissociated substances, and in proportion as these are formed the concentrations of the ions of the original electrolyte are reduced. In order to maintain the condition $c_1c_2 = \text{const.}$ some of the undissociated molecules of the original electrolyte dissociate, thereby making room for the passage of fresh solid into solution. The solubility, therefore, of a sparingly soluble electrolyte must be increased in presence of another which has no ion common with the first. This conclusion also is in harmony with observation.[1] Benzoic acid, for instance, is more soluble in sodium acetate solutions than it is in water, a fact which is brought out by the figures quoted in the following table:—

Concentration of Sodium Acetate.	Solubility of Benzoic Acid.
0·00	0·0289
0·0099	0·0370
0·0198	0·0446
0·0493	0·0643

[1] See Noyes and Chappin, *Journ. Amer. Chem. Soc.*, 1898, 20, 751; Philip, *Journ. Chem. Soc.*, 1905, 87, 987; 1909, 95, 1466.

The numbers given represent in all cases gram-molecules per litre of solution. When sodium acetate is added to a saturated solution of benzoic acid, the two new compounds which may be formed by reactions between the ions are sodium benzoate and acetic acid. The first of these compounds is highly dissociated, like all salts of this type, so that its formation is responsible for the removal of only a small quantity of the $C_6H_5.COO'$ ions. Acetic acid, on the other hand, is a feebly dissociated compound, and its formation means a relatively complete removal of the hydrogen ions. This leads to a big disturbance of the equilibrium between benzoic acid and its ions, to the dissociation of the benzoic acid molecules, and to the replacement of these by fresh solid passing into solution. If sodium chloride were added instead of sodium acetate, the effect on the solubility of benzoic acid would be very slight indeed, because hydrochloric acid is highly dissociated compared with acetic acid. On similar lines intelligible explanations can be given of such facts as that silver acetate is soluble in nitric acid, and that magnesium hydroxide is more soluble in solutions of ammonium chloride (or the chloride of any weak base) than in pure water.

The Law of Mass Action in Immunochemistry.[1]—Within recent years the nature of the relationship between toxins and antitoxins has attracted much attention. The work of Ehrlich and others has shown that the addition of an antitoxin to the corresponding toxin resembles generally the neutralisation of an acid by an alkali, but the fact has emerged also that the amount of toxin neutralised is not proportional to the

[1] See Arrhenius, *Immunochemistry;* also Michaelis in Koranyi and Richter's *Physikalische Chemie und Medizin*, vol. ii.

amount of antitoxin added. The process is therefore not strictly analogous to the neutralisation of a strong acid by a strong base, but rather to that of a weak acid by a weak base. In the latter case the hydrolytic dissociation of the salt interferes with the normal course of neutralisation, and in a mixture containing equivalent quantities of a weak acid and a weak base there is still free acid and free base. These are in reversible equilibrium with the salt, thus: $AB + H_2O \rightleftarrows HA + BOH$. To such a reversible equilibrium the law of mass action may be applied, and it follows that by adding excess of the acid the concentration of the free base is diminished, but only gradually.

The fact that in a solution containing equivalent quantities of a weak base and a weak acid there is free base and free acid is brought out by a study of the neutralisation of ammonia by boric acid.[1] Free ammonia is a hæmolytic agent, that is, acts on red blood corpuscles so as to bring about the escape of the hæmoglobin; boric acid, on the other hand, exerts no appreciable hæmolytic action. The gradual neutralisation of ammonia by boric acid is therefore marked by decreasing hæmolytic activity, and the toxicity (in relation to red blood corpuscles) of a solution containing both ammonia and boric acid may in fact be taken as a measure of the free ammonia which it contains. Since the addition of an exactly equivalent quantity of hydrochloric acid to sodium hydroxide solution completely removes the hæmolytic effect of the latter, it might perhaps be expected that the addition of an equivalent quantity of boric acid to ammonia would give a mixture which is non-toxic in regard to red blood corpuscles. This, however, is not the case, as appears from the

[1] See Arrhenius and Madsen, *Zeit. physikal. Chem.*, 1903, 44, 7.

data recorded in the accompanying table. The figures

n.	Toxicity.
0	100
0·17	85
0·33	69
0·67	43
1·0	25
1·33	20
1·67	13
2·0	10

in the second column represent the toxicity (deduced from the hæmolytic power) of solutions of 1 equivalent of ammonia, to which n equivalents of boric acid have been added. It is evident that a solution in which there are equivalent quantities of ammonia and boric acid still contains free ammonia, and that addition of excess of boric acid only gradually reduces the concentration of the free base.

There is a considerable amount of evidence available which shows that in a neutral mixture of a toxin and its antitoxin a certain proportion of each exists in the free state. There is, for instance, the work done by Craw[1] on the lysin obtained from cultures of *Bacillus megatherium*. This lysin passes through a gelatin filter, whereas the corresponding antilysin is kept back. Making use of this difference between the two bodies, Craw was able to show that both neutral mixtures[2] and those with excess of antilysin contain free lysin, also that both neutral mixtures and those with excess of lysin contain free antilysin. Neutral mixtures, therefore, of lysin and antilysin contain both substances to some extent in the free state, and the question arises whether they are in reversible equilibrium with some compound formed by the union of lysin and antilysin.

[1] *Proc. Roy. Soc.*, B, 1905, 76, 179.

[2] Mixtures, that is, which did not hæmolyse in the standard time.

On the question of the reversibility of the toxin-antitoxin reaction the evidence is somewhat conflicting. Craw finds that the reaction between megatherium lysin and antilysin is reversible when excess of antilysin is present, but, on the other hand, the Danysz phenomenon may be quoted (see p. 215).[1] Danysz found that the toxic properties of a mixture of diphtheria toxin and antitoxin depend on the manner in which they are mixed. Suppose A and T are quantities of antitoxin and toxin such that when A is added to T all at once the mixture is innocuous; then it is found that if A is added to T at intervals, a portion at a time, the resulting mixture is toxic. This observation is difficult to reconcile with the view that there is a true reversible equilibrium between toxin and antitoxin.[2] Further arguments against the view that the toxin-antitoxin reaction is strictly reversible have been brought forward by Nernst and others.[3]

Considerable difference of opinion exists also on the question how far toxins and antitoxins are in a state of true solution. Some regard them purely as colloids, even suspension colloids, and consider that the relation between them is one of adsorption equilibrium. The treatment of the toxin-antitoxin relationship from this point of view has been already illustrated at the close of the previous chapter in reference to the phenomenon of agglutination.

Arrhenius, on the other hand, has found that diphtheria toxin and antitoxin, tetanolysin and antitetanolysin, have a definite power of diffusion, and may be regarded as in a state of true solution. He considers that the equilibrium between a toxin and its antitoxin is reversible

[1] See *Journ. Hygiene*, 1907, **7**, 501.
[2] See, however, Arrhenius, *Journ. Hygiene*, 1908, **8**, 1.
[3] *Zeit. Elektrochem.*, 1904, **10**, 377, 783.

in the ordinary sense, and that therefore the law of mass action may be applied. In various cases the neutralisation of a toxin by its antitoxin has been investigated from this point of view, and the course of neutralisation is found to be in harmony with an equilibrium formula similar to that which represents the neutralisation of ammonia by boric acid. In the case, for instance, of tetanolysin, the following formula was found to apply: $c_1c_2 = Kc^2$, where c_1, c_2, and c are the quantities of free lysin, free antilysin, and bound lysin respectively, and $K = 0{\cdot}115$ at 20°. The quantity of free lysin in any mixture was deduced from its hæmolytic power. How far the experimental figures are in harmony with the foregoing formula will be seen from the following table, in which n is the added quantity of antilysin:—

n.	c_1 found.	c_1 calc.
0	100	100
0·05	82	82
0·1	70	66
0·15	52	52
0·2	36	38
0·3	22	23
0·4	14·2	13·9
0·5	10·1	10·4
0·7	6·1	6·3
1·0	4·0	4·0
1·3	2·7	2·9
1·6	2·0	2·5
2·0	1·8	1·9

There can be no doubt that there is remarkable agreement between the observed and calculated values for the quantity of free lysin, and the formula $c_1c_2 = Kc^2$ evidently represents the actual numerical relationship between the quantities involved. On this ground Arrhenius draws the conclusion that the reaction between toxin and antitoxin is to be represented as 1 mol. toxin + 1 mol.

antitoxin $\rightleftarrows$ 2 mols. toxin-antitoxin compound. In view, however, of the doubts which exist as to the legitimacy of applying the law of mass action to the toxin-antitoxin reaction, the foregoing conclusion must be accepted with reserve.[1]

[1] The discussion of Ehrlich's views and the exposition of his side chain theory lie beyond the scope of this volume.

CHAPTER XIII

THE VELOCITY OF CHEMICAL REACTION

General.—In the foregoing chapter the velocity with which a reversible reaction $A+B \rightleftarrows C+D$ proceeds to its condition of equilibrium has been conceived as the resultant of two component velocities, one the velocity with which A and B react to form C and D, the other the velocity with which C and D react to form A and B. At the point of equilibrium these velocities are equal, the amount of change resulting from the forward reaction per unit of time exactly balancing the amount of change which results from the back reaction. If the reaction is such that the equilibrium position is almost at one extreme, say, at that represented by the right-hand side of the equation $A+B \rightleftarrows C+D$, then the back reaction is negligible in comparison with the forward reaction except when the equilibrium is nearly reached; that is, the velocity of the reaction for the greater part of its course is simply the velocity with which A and B react to form C and D. On the basis of the law of mass action, therefore, the velocity of the reaction at any moment, supposing that it takes place in a homogeneous system, is proportional to the product of the molecular concentrations of A and B at that moment. If a and b represent the molecular quantities of A and B which were mixed initially, and if after an interval of time t the molecular quantity of C and D formed is x, then the velocity V of the reaction at this interval from the start will be given by

$V = k_1(a-x)(b-x)$. But the velocity of the reaction may be defined as the rate at which x is increasing with the time, $-\frac{dx}{dt}$, as it is put in the language of the differential calculus. The formula therefore which, on the basis of the law of mass action, ought to represent the rate of the change $A+B \rightleftarrows C+D$, when the change proceeds until either A or B has practically disappeared, is $\frac{dx}{dt} = k_1(a-x)(b-x)$.

Inversion of Sucrose: a Unimolecular Reaction.—A common example of a reaction of the type $A+B \rightleftarrows C+D$, one too which fulfils the condition that the reaction shall proceed until either A or B has practically disappeared, is the inversion of sucrose. The change which occurs in the inversion of sucrose may be represented as

$$C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6,$$

for although the change takes place with appreciable velocity only in the presence of a catalytic agent, such as hydrochloric acid, yet the latter is found unaltered when the reaction is over. Since the inversion is carried out in aqueous solution the formula $\frac{dx}{dt} = k_1(a-x)(b-x)$ may be simplified, for in this case the water which actually disappears in the reaction is a very small fraction of the total water present;[1] x may therefore be neglected in comparison with b, and we have

$$\frac{dx}{dt} = k_1(a-x)b = k(a-x), \text{ where } k = k_1 b.$$

Integration of the equation $\frac{dx}{dt} = k(a-x)$ leads to the formula $k = \frac{1}{t}\log_e \frac{a}{a-x}$, in which, as already indicated, a is the quantity of sucrose originally present, and $a-x$

[1] Suppose, for instance, that a solution containing 171 grams of sucrose per litre is considered. In 1 litre of this solution there is 877 grams of water, whereas the quantity of water combining with the 171 grams of sucrose during inversion is only 9 grams

is the quantity still to be inverted after an interval t from the start. Any method which permits a relatively rapid determination of the quantity of sucrose in the inverting solution at a given time enables us to test the applicability of this formula, but the only method practically employed in studying the rate of inversion of sucrose is that which depends on the use of the polarimeter. It is well known that a solution of sucrose has a + rotation, whereas the completely inverted solution has a – rotation; further, the change in rotation from the initial angle a_0 to the final angle a_∞, observed after inversion is complete, is a measure of the total quantity of sucrose undergoing change. Similarly, if a is the angle of rotation observed for the solution after an interval t from the start, the difference between a and a_∞ is a measure of the sucrose which has still to be inverted after time t. If, then, $a_0 - a_\infty$ is taken as a measure of a, $a - a_\infty$ is a measure of $a - x$ in the same units; hence $\frac{a}{a-x} = \frac{a_0 - a_\infty}{a - a_\infty}$. The velocity formula may therefore be altered to read $k = \frac{1}{t} \log_e \frac{a_0 - a_\infty}{a - a_\infty}$. The inversion may be allowed to take place in the tube of the polarimeter itself, provided that a constant temperature is maintained by a water-jacket. It is a matter of common experience that the temperature coefficient of a chemical reaction is high, hence in any experimental study of the applicability of the velocity formula care must be taken to ensure a constant temperature. When a solution of sugar containing acid is kept in a suitably jacketed polarimeter tube, the knowledge of the angle of rotation determined at definite intervals enables us to follow the course of the change and to evaluate k for each point. The data in the following table show how far the actual course of inversion corresponds with the velocity formula:—

Inversion of Sucrose at 25° by 0·5N HCl.

t in minutes.	Angle of Rotation.	$k=\frac{1}{t}\log_{10}\frac{a_0-a_\infty}{a-a_\infty}$.
0	+25·16°	...
56	16·95°	0·00218
116	10·38°	0·00218
176	5·46°	0·00219
236	1·85°	0·00219
371	−3·28°	0·00221
∞	−8·38°	...

It ought to be noted that the expression which has been evaluated in the last column is $\frac{1}{t}\log_{10}\frac{a_0-a_\infty}{a-a_\infty}$, instead of $\frac{1}{t}\log_e\frac{a_0-a_\infty}{a-a_\infty}$. But, obviously, if the values of the former expression are constant, the values of the latter must be so also. The figures in the last column are very satisfactorily constant, and the mean value 0·00219 may be taken as a measure of the velocity of inversion of sucrose under the conditions specified, viz. at 25° and in presence of 0·5N HCl. The variation in the velocity coefficient with temperature and with the concentration of the acid will be discussed later.

Reactions, such as the inversion of sucrose, in which the concentration of one substance only is undergoing change, are known as *unimolecular* reactions. The course of all changes of this description is expressed by the formula $k=\frac{1}{t}\log\frac{a}{a-x}$. All hydrolytic changes which take place in aqueous solution belong to this category, as, for instance, the reaction

$$CH_3.COOCH_3+H_2O\rightleftarrows CH_3.COOH+CH_3OH;$$

under the influence of an acid this change goes completely from left to right, and the course of the change is represented by the foregoing formula.

The rate of hydrolysis of methyl acetate, like the

rate of sugar inversion, is within certain limits proportional to the concentration of the hydrogen ions present. A determination, therefore, of the rate of hydrolysis of methyl acetate as influenced (1) by any feebly acid fluid, (2) by dilute hydrochloric acid containing a known quantity of hydrogen ions, permits the calculation of the hydrogen ion concentration in the said fluid. In this way information can be gained which a mere titration cannot give, for by the latter operation we determine only the total acidity of the fluid, and obtain no indication of the ratio of ionised acid to total acid. By a study, however, of the influence of the fluid in question on the velocity of hydrolysis of methyl acetate the extent of the ionisation is ascertained. This method has been employed, for instance, in the investigation of the acidity of the contents of the stomach.[1]

Further Discussion of the Formula for a Unimolecular Reaction.—The formula $k=\frac{1}{t}\log_e\frac{a}{a-x}$, which represents the course of a unimolecular reaction, has in the foregoing pages been reached by purely mathematical operations. Although this is in one sense absolutely satisfactory, it is worth while to consider a little more in detail what is involved in the formula, and to endeavour to translate the mathematical expressions into terms which may be more capable of direct interpretation. For this purpose it will be convenient to refer specially to the inversion of sucrose; any conclusions established for this typical unimolecular reaction may be extended to cover other reactions which belong to the same type.

The fundamental formula for the inversion of sucrose

[1] See Moore, *Proc. Roy. Soc.*, B, 1905, **76**, 138.

is, as already quoted, $\frac{dx}{dt} = k(a-x)$, where dx is the amount of change in the interval of time dt, $a-x$ is the amount of unchanged sugar at the moment, and k is a constant. If the formula is written $\frac{dx}{a-x} = kdt$, it is evident that for a given interval of time the amount of change must be a constant fraction of the unchanged sugar present. Experimental work on the rate of inversion of sucrose confirms this conclusion, as appears from consideration of the data in the following table.[1] A sucrose solution containing 17·1 grams

Time.	α
0	+21·55°
15	20·40°
120	13·75°
135	12·95°
225	8·62°
240	8·02°
∞	−7·18°

of sugar per 100 cub. cm. was inverted at 20° under the influence of hydrochloric acid, and the progress of the change was followed by determining the rotation (α) of the solution from time to time. The decrease in rotation during the first 15 minutes, namely, 1·15°, is a measure of the amount of change which has taken place during that interval. The average rotation of the solution for the same interval of 15 minutes may be taken as $\frac{21·55+20·40}{2} = 20·97$, and a measure of the mean amount of unchanged sucrose present during this interval is given by $20·97+7·18=28·15$. The ratio of the amount of change in the first 15 minutes to the unchanged sucrose present may therefore be taken as $\frac{1·15}{28·15} = 0·041$. If, now, the interval

[1] Armstrong and Caldwell, *Proc. Roy. Soc.*, A, 1905, 74, 199.

between 120 and 135 minutes is considered, the amount of change measured by the decrease of rotation which is found for that interval is 0·80. The average rotation of the solution during these 15 minutes may be taken as $\frac{13{\cdot}75+12{\cdot}95}{2} = 13{\cdot}35$, and a measure of the mean amount of unchanged sucrose present is given by $13{\cdot}35+7{\cdot}18=20{\cdot}53$. The ratio of the amount of change in those 15 minutes to the unchanged sucrose present is therefore $\frac{0{\cdot}80}{20{\cdot}53}=0{\cdot}039$, practically the same value as for the first 15 minutes of the inversion. Again, if the data for a still later interval (225–240 minutes) are considered, the ratio of the amount of change to the unchanged sucrose present works out to 0·039. The experimental data, therefore, are in harmony with the statement that in a unimolecular reaction the amount of change in a given short interval is a constant fraction of the unchanged material present.

Another result which can be read out of the formula for the inversion of sucrose becomes clear when it is written in the form $k=\frac{1}{t}\log_e\frac{1}{1-\frac{x}{a}}=\frac{1}{t}\log_e\frac{1}{1-y}$, y representing the fraction of the sucrose which has undergone change up to time t. Since k is a constant for this reaction at a given temperature, it follows that for any selected value of t, y must have a definite value; the fractional amount therefore of the sucrose inverted in a given time is independent of a, *i.e.* independent of the amount of sucrose initially present. The validity of this conclusion may be tested by comparing the values of the velocity coefficient obtained in experiments carried out with varying quantities of sucrose: the theory requires that the velocity coefficients so obtained should be equal. How far this is the case will appear

from the figures in the following table.[1] The numbers

Gram-mols. of Sucrose.	k_1.	k_2.
0·25	560	504
0·5	622	510
0·75	698	513
1·0	770	521

in the first column represent the quantity of sugar taken, and the numbers under k_1 in the second column are the mean values of the velocity coefficient for each experiment, the sugar solution containing hydrochloric acid in each case to the extent of 1 gram-molecule per litre. Instead of being constant, as the theory requires, the values of k_1 increase markedly as the sucrose concentration increases. It has, however, been pointed out that as the sucrose concentration increases, the amount of water present in 1 litre of sucrose solution diminishes to a considerable extent; the concentration of the hydrochloric acid, therefore, which acts as the catalytic agent, is not constant throughout, and hence the values for k_1 cannot fairly be compared. If the proportion of acid to water is kept constant, that is, if the sucrose and the gram-molecule of hydrogen chloride are in each case dissolved in 1000 grams of water,[2] then the values recorded under k_2 are obtained for the velocity coefficient. The extent to which these values vary with increasing quantity of sucrose is comparatively slight, and they may therefore be regarded as confirming the statement that the fractional amount of sucrose inverted in a given time is independent of the amount of sucrose originally present.

Saponification of an Ester by an Alkali. A Bimolecular Reaction.—When sodium hydroxide is added

[1] Caldwell, *Proc. Roy. Soc.*, A, 1906, 78, 287.

[2] For this method of preparing solutions, see p. 48.

to a solution of ethyl acetate, the ester is gradually decomposed into sodium acetate and ethyl alcohol. The progress of the decomposition is marked by the decreasing alkalinity of the solution, and therefore by extracting a measured portion from time to time, and titrating with standard acid, the velocity of the reaction can be quantitatively studied. The saponification of the ester may be represented by the equation

$$CH_3.COOC_2H_5 + NaOH = CH_3.COONa + C_2H_5OH,$$

from which it will be seen that in this case the concentrations of *two* substances undergo change during the reaction. The law of mass action applied to this case leads to the formula $\frac{dx}{dt} = k(a-x)(b-x)$, where a and b are the initial quantities of ester and alkali, and x is the quantity of sodium acetate produced after an interval t from the start. If this equation is integrated, we find the velocity constant $k = \frac{1}{(a-b)t} \log_e \frac{b(a-x)}{a(b-x)}$.

The mathematical expression which represents the course of the reaction becomes simpler if the ester and alkali are taken in equivalent quantities, *i.e.* if $a = b$. In this case $\frac{dx}{dt} = k(a-x)^2$, the integration of which gives the formula $k = \frac{1}{t} \frac{x}{a(a-x)}$. How far the facts are in harmony with this formula may be seen from the figures in the following table.[1] The solution used was $\frac{N}{40}$ in relation both to ester and sodium hydroxide, and during the reaction the mixture was kept at 24·7°. The figures recorded under $a-x$ are the volumes of a standard hydrochloric acid required to neutralise exactly 10 cub. cm. of the reaction mixture.

[1] Arrhenius, *Zeit. physikal. Chem.*, 1887, 1, 110.

t (min.).	$a-x$.	$ka=\frac{1}{t}\frac{x}{(a-x)}$.
0	8·04	...
4	5·30	0·129
6	4·58	0·126
8	3·91	0·132
10	3·51	0·129
12	3·12	0·131
15	2·74	0·129
20	2·22	0·131

The numbers given in the last column for ka are very satisfactorily constant, and confirm the application of the law of mass action to a bimolecular reaction.

As indicated on p. 168, the rate of inversion of sucrose and the rate of saponification of an ester may be utilised in ascertaining the concentration of hydrogen or hydroxyl ions respectively in any solution. It must, however, be borne in mind that there is an essential difference between the two reactions. In the saponification of an ester by an alkali, the alkalinity of the reaction mixture diminishes as the reaction proceeds, *i.e.* the hydroxyl ions disappear. In the inversion of sucrose, on the other hand, the acid present does not enter into the products of the reaction, and the quantity of the acid or, in other words, the concentration of the hydrogen ions remains the same throughout the course of the change. The influence of acids in promoting the inversion of sucrose is an example of catalytic action, the general characteristics of which must be considered in some detail.

Catalysis.—It is a well-known fact that a chemical change which of itself proceeds with extreme slowness may be greatly accelerated in presence of some apparently foreign substance, the other conditions being unaltered. The amount of this foreign substance may be extremely small, it may not take any obvious part in the chemical change, it may be quantitatively recoverable at the end

of the change, and yet the rate of the chemical reaction may be markedly altered. This phenomenon has been known for a long time, and is familiar to the chemist as 'catalysis.' It is of the greatest importance in relation to the chemical changes which take place in the living organism, for there we find reactions occurring easily and smoothly, which, apart from the organism, are exceedingly sluggish and difficult to bring about. The 'catalytic agents' or 'catalysts' which promote the processes of metabolism in the living organism belong to the class of enzymes, and it will presently appear that from the quantitative as well as the qualitative point of view there is a close analogy between enzymes and inorganic catalysts.

A quantitative study of catalysis is possible only on the basis of the law of mass action. In the velocity coefficient, as already explained for the inversion of sucrose, we have a quantitative expression for the rate of a chemical change under given conditions. For a given reaction, therefore, which is catalytically accelerated, the value of the velocity coefficient at a given temperature is a measure of the efficiency of the catalyst, and by comparing the values obtained for the velocity coefficient in different experiments one can ascertain how the efficiency of the catalyst varies with the conditions under which it works, and how the efficiency of one catalyst compares with that of another working under the same conditions.

Characteristics of Inorganic Catalysts.—One of the most striking features about a catalyst is that its quantity may be so minute compared with the quantities of the main reacting substances. An illustration of this is furnished by the influence of molybdic acid on the rate of the reaction between hydrogen peroxide and hydriodic acid. Brode[1] has shown that the velocity of interaction

[1] *Zeit. physikal. Chem.*, 1901, 37, 257.

between these substances in $\frac{N}{100}$ solution is more than doubled by the addition of molybdic acid, even in the proportion of 1 molecule molybdic acid to 1 million litres of solution.

Another point which has been established by the quantitative study of inorganic catalysts is that, as a rule, the activity of the catalyst at the end of the reaction which it has accelerated is unimpaired. In this connection reference may be made again to the fact that the acid used to effect the inversion of sucrose does not appear in the products of the reaction, and is present in undiminished quantity when the inversion is complete. Another illustration of the same principle is furnished by the behaviour of colloidal platinum in promoting the union of hydrogen and oxygen. In the course of some experiments made by Bredig,[1] it was found that when electrolytic gas is shaken with a colloidal solution of platinum at the ordinary temperature, there is a fairly rapid decrease in volume owing to the union of hydrogen and oxygen. In one case where 2·5 cub. cm. of a colloidal platinum solution (containing 0·17 milligram platinum) was shaken with electrolytic gas, the results recorded in the following table were obtained:—

Time in Minutes.	Decrease in the Volume of Gas.	Rate of Decrease in cub. cm. per Min.
10	17·8 cub. cm.	1·78
20	35·8 „	1·80
30	54·8 „	1·90
40	72·4 „	1·76
50	90·2 „	1·78

The average of the figures in the last column is a measure of the catalytic efficiency of the colloidal platinum in the early stages of its activity. The same colloidal platinum was shaken intermittently during fourteen days with electrolytic gas, about 10 litres of which disappeared

[1] *Zeit. physikal. Chem.*, 1899, 31, 258; see also Ernst, *ibid.*, 1901, 37, 448.

in this time under the influence of the catalyst. The actual rate of disappearance of the gas at the end of the fourteen days was then definitely measured, with the following results:—

Time in Minutes.	Decrease in the Volume of Gas.	Rate of Decrease in cub. cm. per Min.
10	20·2 cub. cm.	2·02
20	38·9 „	1·87
30	58·4 „	1·95
40	78·1 „	1·97
50	98·2 „	2·01

The average of the figures in the last column is a measure of the catalytic efficiency of the colloidal platinum after it has exerted its activity for a considerable period. It is obvious that the efficiency is unimpaired; the average rate of decrease in the volume of the electrolytic gas is even slightly greater at the end than at the beginning.

So far, we have conceived a catalyst as a substance which merely accelerates a chemical reaction, and does not appear in the products of the reaction. If this is so, the final state of the reactive system must be independent of the catalyst; the state of equilibrium finally reached between the reacting substances must be the same whether the catalyst has been present or not. In other words, the catalyst influences only the rate at which the condition of equilibrium is reached, not the position of equilibrium itself. The validity of this conclusion can be tested more suitably in connection with a reversible reaction, for in such a case the position of equilibrium is defined by the value of the equilibrium constant. As already shown, the equilibrium constant for a reversible reaction is equal to the ratio $\frac{k_1}{k_2}$, where k_1 is the velocity coefficient of the forward reaction, and k_2 is that of the back reaction. If, then, the position of equilibrium is not affected by the presence of a catalyst,

it follows that the forward and the back reactions must be accelerated in the same proportion. This has been shown to be actually the case in connection with the catalytic action of acids on the velocities of esterification and hydrolysis of an ester.

That the position of equilibrium for a reversible reaction is independent of the nature and amount of catalyst which has been used to accelerate the establishment of equilibrium is shown clearly by Turbaba's study[1] of the relationship between aldehyde—$CH_3.CHO$—and paraldehyde—$(CH_3.CHO)_3$. The equilibrium mixture of these two substances at 50·5° contains 33·9 per cent. aldehyde and 66·1 per cent. paraldehyde. The conversion of paraldehyde into the equilibrium mixture is accompanied by an expansion, and the course of the change may therefore be followed in a dilatometer. Various substances in varying amount may be employed to accelerate the change, but the difference between the initial and the equilibrium volumes, as shown by the figures below, is the same in all cases; that is, the position of equilibrium is independent of the nature and amount of the catalyst:—

Catalyst.	Per Cent. Catalyst.	Percentage Increase of Volume.
Sulphur dioxide . . .	0·08	8·20
„ . . .	0·07	8·34
„ . . .	0·002	8·19
Zinc sulphate	2·7	8·13
Hydrochloric acid . . .	0·13	8·13
Oxalic acid	0·52	8·27
Phosphoric acid . . .	0·54	8·10

Another point of great interest is the relationship between the value of the velocity coefficient for a given reaction at a given temperature and the concentration of the catalyst. In a great many cases the relation-

[1] See *Zeit. physikal. Chem.*, 1901, 38, 505.

ship is a linear one, that is, the velocity coefficient is directly proportional to the concentration of the catalyst. The rate of inversion of sucrose by acids, for instance, is proportional to the concentration of the hydrogen ions, provided that this concentration is low, and on the basis of this proportionality it is possible to calculate the velocity of inversion by dilute acetic acid from the velocity observed with dilute hydrochloric acid. Again, the acceleration of the reaction between hydriodic acid and hydrogen peroxide by molybdic acid is proportional to the concentration of the latter.[1]

In other cases the relationship between reaction velocity and concentration of catalyst is not a linear one. The influence of colloidal platinum in promoting the decomposition of hydrogen peroxide is a case in point.[2] The course of this reaction, it may be explained, is easily followed by extracting a definite volume of the reaction mixture from time to time and titrating with a dilute solution of potassium permanganate. The volume of permanganate required for each extract is a measure of the undecomposed hydrogen peroxide present in the reaction mixture at the time of the extraction. For a given temperature and a given concentration of colloidal platinum the course of the decomposition is represented by the formula for a unimolecular reaction; this appears from the figures in the accompanying table,

t min.	$a-x$.	k.
0	22·3	...
10	13·6	0·022
20	8·05	0·022
30	4·6	0·023
35	2·8	0·022

where the numbers under $a-x$ represent cub. cm. of permanganate required for a given volume of reaction

[1] Brode, *loc. cit.*

[2] See Bredig and von Berneck, *Zeit. physikal. Chem.*, 1899, **31**, 258.

mixture, and those under k are the values of the velocity coefficient calculated for a unimolecular reaction. The mean value obtained for k in different experiments varies with the concentration of the platinum in the manner shown by the following figures. From these

Platinum Concentration.	k.
21×10^{-6}	0·072
$10{\cdot}5 \times 10^{-6}$	0·024
$5{\cdot}2 \times 10^{-6}$	0·0084
$2{\cdot}6 \times 10^{-6}$	0·0027

it appears that when the concentration of the catalyst is doubled, the velocity of decomposition is trebled.

Enzymes as Catalysts.[1]—In many respects enzymes resemble inorganic catalysts. To begin with, there is the same striking contrast between the small quantity of the enzyme and the extent of the chemical change which it brings about. O'Sullivan and Tompson [2] refer to a sample of invertase which had induced the inversion of one hundred thousand times its own weight of sucrose and was still active. Senter, in the course of experiments on hæmase,[3] an enzyme present in the blood, found that when 100 cub. cm. of a solution of blood (obtained by adding 1 cub. cm. of blood to 1000 cub. cm. of water) are mixed with 100 cub. cm. of a hundredth molar solution of hydrogen peroxide, the whole of the latter is decomposed in 5 minutes, although the solution without any enzyme exhibits no appreciable decomposition in 12 hours.

Enzymes resemble inorganic catalysts also in that, where the reaction involved is a reversible one, they

[1] For a detailed discussion of this subject see *The Nature of Enzyme Action*, by W. M. Bayliss.

[2] *Journ. Chem. Soc.*, 1890, 57, 834.

[3] *Zeit. physikal. Chem.*, 1903, 44. 257.

promote both the direct and the reverse changes. An instance of this is furnished by the action of lipase on the esters of the lower fatty acids. If this enzyme is allowed to act on ethyl butyrate in presence of water, partial hydrolysis into butyric acid and ethyl alcohol takes place; while if it is allowed to act on an aqueous mixture of butyric acid and ethyl alcohol, a certain quantity of the ester is formed.[1] The action of an enzyme, however, on the products of a reaction may not be strictly the reverse of its effect on the forward reaction, for it has been found[2] that maltase, the enzyme which hydrolyses maltose into dextrose, exerts a synthetic action on dextrose, producing not maltose, but *iso*-maltose.

In certain cases the course of a reaction induced by an enzyme is in harmony with the law of mass action. The decomposition of hydrogen peroxide under the influence of hæmase[3] may be taken as an example of this. The course of the decomposition is followed in the same way as already described for the catalysis of hydrogen peroxide by colloidal platinum; that is, a definite volume of the reaction mixture is taken out from time to time and titrated with dilute potassium permanganate solution. That the course of the change is in harmony with the law of mass action is shown by the following table, the figures under $a-x$ re-

t (min.).	$a-x$.	k.
0	11·0	...
5½	8·7	0·0194
10	7·4	0·0172
20	4·8	0·0180
30	3·0	0·0188
50	1·3	0·0185

[1] Kastle and Loevenhart, *Amer. Chem. Journ.*, 1900, 24, 491.

[2] Croft Hill, *Journ. Chem. Soc.*, 1898, 73, 634; 1903, 83, 578; Emmerling, *Ber.*, 1901, 34, 600, 2206, 3810; Armstrong, *Proc. Roy. Soc.*, B, 1905, 76, 592.

[3] Senter, *loc. cit.*

presenting the volume of dilute permanganate solution required for 25 cub. cm. of the reaction mixture, and those under k being the values of the velocity coefficient calculated for a unimolecular reaction. The initial concentration of the hydrogen peroxide was in this case $\frac{1}{400}$th molar, and the experiments were carried out at 0° C. The constancy of the numbers in the last column shows that the catalysis of hæmase follows the course of a unimolecular reaction. Further, it was found by Senter that, for hydrogen peroxide solutions between $\frac{1}{300}$th and $\frac{1}{1000}$th molar concentration, the value of the velocity coefficient is independent of the initial concentration of the hydrogen peroxide; this also supports the view that the action of hæmase on hydrogen peroxide is in harmony with the law of mass action (see p. 282). It appears too that, at least in very dilute solutions of hydrogen peroxide, the velocity of decomposition is proportional to the concentration of the enzyme.

In many respects there is a close parallelism between the decomposition of hydrogen peroxide by colloidal platinum and the decomposition of the same substance under the influence of hæmase. This parallelism extends also to the effect of certain 'poisons' in paralysing the activity of the two catalysts,[1] so much so that Bredig has described colloidal platinum as an 'inorganic ferment.'

The catalysis of hydrogen peroxide by hæmase has been referred to as a case in which enzyme action conforms to the law of mass action, and in which the enzyme behaves very similarly to an inorganic catalyst. The close study, however, of many other cases has shown that very frequently, owing to the operation of

[1] Bredig and von Berneck, *Zeit. physikal. Chem.*, 1899, 31, 258; Senter, *loc. cit.*, and *Proc. Roy. Soc.*, 1905, 74, 201.

various factors, the course of a reaction which takes place under the influence of an enzyme deviates considerably from what we should expect on the basis of the law of mass action. A brief discussion of some of these factors may be found useful.

Some Peculiarities of Enzyme Action.—As an instance of an enzyme reaction deviating from the course marked out by the law of mass action, the inversion of sucrose by invertase may be quoted. This change has been studied quantitatively by A. J. Brown,[1] and the following table embodies the results of one of his experiments. In this particular case 25 cub. cm. of invertase solution were added to 500 cub. cm. of a 9·48 per cent. sucrose solution, and the mixture was kept at 30°. Portions were extracted from time to time, and from the observed rotation for each sample the extent to which inversion had proceeded at the time of extraction was deduced; the figures under x represent the fraction of the total sucrose which had undergone inversion by time t. The numbers in the last column, instead of being constant, as they ought to be if the inversion proceeds in conformity with the law of mass action, exhibit a marked and regular increase.

t min.	x.	$\frac{1}{t}\log\frac{1}{1-x}$.
30	0·265	0·00445
64	0·509	0·00483
120	0·794	0·00571
180	0·945	0·00698
240	0·983	0·00737

The departure from the law of mass action becomes still clearer when experiments are made in which a

[1] *Journ. Chem. Soc.*, 1902, 81, 373. See also Henri, *Zeit. physikal. Chem.*, 1901, 39, 194.

constant amount of invertase is allowed to act for a given time on varying amounts of sucrose in a constant volume of solution. According to the law of mass action, the fraction of the sucrose inverted in the given time ought to be the same in all cases, independent, that is, of the initial quantity of sucrose present. How far this requirement of the law of mass action is fulfilled will be seen from the accompanying table:—

Grams Sucrose per 100 cub. cm.	Grams Sucrose Inverted in 60 min.	Fraction of Sucrose Inverted in 60 min.
4·89	1·230	0·252
9·85	1·355	0·138
19·91	1·355	0·068
29·96	1·235	0·041

It is clear that the enzyme, instead of inverting a *constant fraction*, has inverted an approximately *constant weight* of sucrose in the given time. On the other hand, if the quantity of sucrose is relatively much smaller than in the cases recorded in the foregoing table the law of mass action is fulfilled, in that the weight of sucrose inverted in a given time is always the same fraction of the weight taken initially. This appears from the following figures:—

Grams Sucrose per 100 cub. cm.	Grams Sucrose Inverted in 60 min.
1·0	0·249
0·5	0·129
0·25	0·060

Other cases in which it has been found that the amount of change induced by an enzyme is, for at least a portion of the change, a linear function of the time, are the hydrolysis of starch by diastase,[1] and the hydrolysis of milk sugar by lactase.[2]

[1] H. T. Brown and Glendinning, *Journ. Chem. Soc.*, 1902, 81, 388.

[2] E. F Armstrong, *Proc. Roy. Soc.*, 1904, 73, 500.

In connection with the former of these cases it has been shown that it is only the earlier portion of the time-curve which is linear, the later portion being logarithmic in character. This is proved by calculating the velocity coefficient $k=\frac{1}{t}\log\frac{1}{1-x}$ (1) for each observation from the start of the reaction, (2) for each observation after the linear portion has been passed, a new starting point being chosen. A comparison of two sets of values of k obtained in this manner is given in the following table, which refers to the hydrolysis of a 3 per cent. starch solution by malt extract at 51°–52° :—

Time (min.).	k.	Time in min. from new Starting Point.	k.
10	0·00498		
20	0·00553		
30	0·00590		
40	0·00620	0	...
50	0·00650	10	0·00842
60	0·00690	20	0·00831
70	0·00706	30	0·00821
80	0·00728	40	0·00837
90	0·00730	50	0·00818
100	0·00732	60	0·00807
110	0·00749	70	0·00822
120	0·00762	80	0·00840
130	0·00779	90	0·00855

It will be seen that the values of k in the second column are far from constant, and yet if the first portion of the change is left out of account, practically constant values for the velocity coefficient are obtained. It is permissible to draw the conclusion that the later portion of the change conforms to the law of mass action.

From the foregoing it appears that it is only when the

amount of enzyme is relatively small compared with the amount of carbohydrate that a linear relationship between the time and the amount of change is observed. To regard the occurrence of this linear relationship, however, as something peculiar to enzymes is scarcely correct, for it has subsequently been found that a similar feature, if less distinct, characterises the hydrolysis of sucrose by very dilute acid.[1] When sucrose solutions containing 171 and 342 grams per litre are inverted at 40° by $\frac{N}{500}$ HCl, the values calculated for the velocity coefficient increase during the first portion of the change and then remain constant. In this respect, therefore, there is a close parallelism between acid and enzyme action: in both cases, when the proportion of catalyst is relatively small, the amount of change is to begin with approximately a linear function, and subsequently a logarithmic function, of the time.

Another peculiarity about enzyme action which has been observed frequently, is that the activity of the enzyme does not remain constant throughout the whole course of the change which it induces. Tammann,[2] for instance, found that in the hydrolysis of amygdalin by emulsin the change is incomplete. The failure of the enzyme to effect complete hydrolysis might be attributed to the really reversible character of the process, but this view is untenable, for if more emulsin is added to a mixture in which hydrolysis has come to a standstill, the reaction proceeds further. This shows clearly that the equilibrium reached when emulsin acts on amygdalin is not one which is independent of the enzyme, as would be the case if the emulsin behaved like an inorganic catalyst. The natural conclusion is that the emulsin

[1] Armstrong and Caldwell, *Proc. Roy. Soc.*, 1905, **74**, 195.

[2] *Zeit. physiol. Chem.*, 1892, **16**, 271.

must be put out of action in some way by the products of hydrolysis—a view which finds support in the fact that the action of emulsin on amygdalin is inhibited by the initial addition of benzaldehyde or hydrocyanic acid. This check to the activity of the enzyme cannot, however, be due to its destruction, for when the products of hydrolysis present in an equilibrium mixture are removed, the splitting up of the amygdalin sets in again.

The influence of the products of change on the activity of the enzyme which induces the change is apparent also in the values which are found for the velocity coefficient in the hydrolysis of milk sugar by lactase.[1] In the accompanying table t gives the time in hours from

t.	x.	k.
1	13·7	0·0640
2	22·1	0·0543
3	27·2	0·0460
5	30·0	0·0310
24	51·0	0·0129

the start, x is the percentage of sugar hydrolysed up to time t, and k is the velocity coefficient calculated by the formula for a unimolecular reaction; the solution contained initially 5 grams milk sugar in 100 cub. cm. In contrast to the case of the inversion of sucrose by invertase (see p. 294), the values of k in this case *decrease* as the hydrolysis proceeds, a result that is attributed to the increasing concentration of the products of hydrolysis. It can indeed be shown that the initial addition of galactose materially reduces the rate of hydrolysis of milk sugar by lactase, while glucose and fructose are practically without effect. This appears from the following table, the figures in which, apart from the first column, represent the percentages of

[1] Armstrong, *Proc. Roy. Soc.*, 1904, 73, 500.

milk sugar hydrolysed; the concentration of milk sugar was in each case 5 grams per 100 cub. cm.:—

Time in Hours.	Milk Sugar alone.	Milk Sugar +5 grams Fructose.	Milk Sugar +5 grams Galactose.	Milk Sugar +5 grams Glucose.
4	18·0	18·0	16·0	17·6
22	59·2	59·6	47·4	59·6
28	65·6	65·4	52·0	65·4
69	81·4	80·2	61·6	78·4

The retarding influence of the products of change is therefore a specific influence, depending on some special relationship between the enzyme and the particular hexose which exerts the retarding effect. Further, the activity of the enzyme, according to the investigations of Fischer and others,[1] is determined by the degree of similarity in the configuration of enzyme and substrate (that is, the substance undergoing change under the influence of the enzyme). It is interesting to note, on the other hand, that the hydrolysis of milk sugar by hydrochloric acid is *accelerated* by the addition of glucose or galactose; the products of change exert no specific influence in this case: indeed, the addition of the equivalent quantity of a neutral salt brings about a similar acceleration.

The fermentation of glucose by yeast juice supplies another instance of the more complicated character of enzyme actions as compared with changes which are accelerated by inorganic catalysts. It has been found that the ferment in yeast juice is of itself unable to bring about the alcoholic fermentation of glucose; another body, the 'co-ferment,' as it is called, which is present in yeast juice, is essential to the activity of the ferment.[2] A separation of the ferment and co-ferment is effected

[1] See Armstrong, *Proc. Roy. Soc.*, 1904, 73, 520.

[2] See Harden and Young, *Proc. Roy. Soc.*, B, 1906, 77, 405; 78, 369.

by dialysis; the residue, containing the ferment, and the dialysate, containing the co-ferment, are separately inactive, but when united give rise to fermentation. The inactive residue obtained on dialysis can be rendered active also by the addition of boiled and filtered yeast juice; it follows, therefore, that the co-ferment is not destroyed by boiling. During the process of fermentation the co-ferment disappears, as has been shown by experiments in which a fairly large quantity of the inactive residue from dialysis and a small quantity of boiled yeast juice have been added to a glucose solution. In this case the evolution of carbon dioxide soon comes to an end, but on the addition of a further quantity of boiled juice fermentation is set up again.

The Mechanism of Catalysis.—The phenomena of catalysis generally, and more particularly those of enzyme action, give rise to the question: How does the catalyst exert its influence? In the present state of our knowledge it is impossible to give a complete and satisfactory answer to this question, but it is desirable to indicate some of the main facts which have a bearing on the problem, and some of the suggestions which have been contributed towards its solution.

It will be convenient to start from the suggestion, which has been very generally accepted, that a catalyst is effective because it forms some sort of combination with the substrate. This intermediate compound, it is supposed, then breaks up into the final products of change, the catalyst being liberated. Obviously, if this account of the catalytic change is to give an adequate interpretation of the phenomena, it is necessary to suppose that the formation and decomposition of the intermediate compound together require a much shorter time for their occurrence than the direct change itself.

In favour of the view that combination of some kind takes place between catalyst and substrate there is a considerable amount of evidence. As found by O'Sullivan and Tompson,[1] invertase in the presence of sucrose stands without injury exposure to a temperature 25° higher than it does in the absence of sucrose. Similarly, proteins exert a protective influence over trypsin.[2] More direct evidence of the formation of intermediate compounds has been brought forward by Brode[3] in his study of the accelerating influence of molybdic acid on the reaction between hydrogen peroxide and hydrogen iodide. In this case it can be proved that combination takes place between the molybdic acid and the hydrogen peroxide, with the result that the former is practically converted into permolybdic acid. It is then supposed that the reduction of this substance by hydriodic acid takes place much more rapidly than the reduction of hydrogen peroxide. Other facts in favour of the view that catalysts act by forming intermediate compounds are the occurrence of a linear portion in the time curve for the hydrolysis of sugars by relatively small quantities of the appropriate enzymes (see p. 295), and also the specificity of enzymes. But although we may with some confidence assume the formation of intermediate compounds in enzyme action and catalysis generally, it is quite impossible in the majority of cases to specify the nature of these compounds.

In this connection it must be borne in mind that many catalytic reactions are to be described as non-homogeneous reactions, as, for instance, the union of hydrogen and oxygen under the influence of platinum black. Here the catalyst is solid, whilst the reacting

[1] *Journ. Chem. Soc.*, 1890, 57, 834.

[2] Bayliss and Starling, *Journ. Physiol.*, 1904, 30, 61.

[3] *Zeit. physikal. Chem.*, 1901, 37, 257. See also p. 286.

substances are both gaseous; the system is a 'two-phase' one. Solutions of colloidal platinum and solutions of enzymes, which are colloids, are also to be regarded as two-phase systems, and in such cases there is the possibility of a surface concentration and the formation of adsorption compounds, such as those described in Chapter XI. The combination between catalyst and substrate, according to this view, would be more physical than chemical in type.

In connection with the velocity of reaction in non-homogeneous systems, doubt has been expressed whether in all such cases one is actually measuring the rate of a chemical change. Nernst[1] has pointed out that a reaction in a non-homogeneous system involves, in addition to a chemical change, a diffusion of various substances to and from the common boundary of the two phases. It is therefore evident that where the rate of the chemical change is relatively very great, the observed velocity of reaction may be merely a diffusion velocity. An actual example of this is found in the rate of solution of marble and various metals in acids, and Nernst has suggested that the velocity of decomposition of hydrogen peroxide by colloidal platinum is determined by the rate of diffusion of the peroxide to the surface of the platinum particles. While this may be so, the theory cannot be regarded as applicable to all reactions in non-homogeneous systems.

The Temperature Coefficient of Reaction Velocity.—It is well known that the velocity of a chemical reaction increases very rapidly as the temperature rises, and as a result of this the range of temperature over which quantitative investigation of the velocity of a reaction is possible is comparatively limited. So far as reactions

[1] *Zeit. physikal. Chem.*, 1903, 47, 52; also Brunner, *ibid.*, 56.

in *homogeneous* systems are concerned, it is found that, as a general rule, the velocity is doubled or trebled for a rise of 10° C. Reactions of the most varied character conform to this rule, but the inversion of sucrose by acid will serve as an example. The accompanying table gives the values of k, the velocity coefficient for this reaction, at temperatures between 25° and 55°. These

Temperature.	k.
25°	9·67
40°	73·4
45°	139
50°	268
55°	491

figures are a quantitative expression of the increase of velocity with rise of temperature, and it will be seen that in this case $\frac{k_{T+10}}{k_T}$ lies between 3 and 4. The average, however, of the temperature coefficient for a reaction in a homogeneous system is between 2 and 3.

The value of the temperature coefficient for a *non-homogeneous* reaction is frequently found to be considerably lower. The influence of temperature on the catalysis of hydrogen peroxide by colloidal platinum[1] may be quoted as an instance; in this case $\frac{k_{T+10}}{k_T} = 1{\cdot}7$, while for the catalysis of hydrogen peroxide by hæmase $\frac{k_{T+10}}{k_T} = 1{\cdot}5$. These values are not much greater than the value (about 1·3) we should expect if the rate of reaction were determined by a diffusion velocity alone, and it is therefore probable that in finding the velocity of catalysis in both these cases one is measuring the velocity of a physical process, not of a chemical change.

[1] Bredig and von Berneck, *Zeit. physikal. Chem.*, 1899, 31, 258.

In the case of most enzyme actions, however, the temperature coefficient is considerably higher than that corresponding to a diffusion velocity, and is of the same order as that usually observed for chemical reactions in homogeneous systems.[1]

The relation between temperature and enzyme action is complicated to some extent by the occurrence of a so-called 'optimum' temperature. An example of this is found in the hydrolysis of salicin by emulsin.[2] The velocity of this hydrolysis at various temperatures is represented in the accompanying table, the velocity at

$t°$ C.	Velocity.
0	1·0
20·5	2·3
30·0	5·8
40·2	8·8
50·3	15·8
60·6	13·3
70·0	12·3

0° being taken as unity. In the neighbourhood of 50° there is a point, the optimum temperature, at which hydrolysis proceeds more rapidly than at any other temperature. The existence of such a point must not be taken to indicate the abrogation of the rule that reaction velocity increases rapidly with rise of temperature. The falling off in the velocity at the higher temperatures is due to the destruction of the enzyme, and the diminution in the effective quantity of the catalyst more than counterbalances the increase in the velocity which rise of temperature invariably brings about. All colloidal systems are liable to be affected in a similar way by rise of temperature, and

[1] See Senter, *Journ. Physical Chem.*, 1905, 9, 311.
[2] Tammann, *Zeit. physiol. Chem.*, 1892, 16, 323.

it is therefore not surprising that there is an optimum temperature for the union of hydrogen and oxygen under the influence of colloidal platinum solution. In an experiment described by Ernst,[1] 2 cub. cm. of a solution of colloidal platinum were shaken at 25° with electrolytic gas, and the decrease in volume in 3 minutes was 1·02 cub. cm. When 2 cub. cm. of the same platinum solution were kept at 45° for two hours and then shaken with electrolytic gas at this temperature, the decrease in volume in 3 minutes was 1·41 cub. cm. The corresponding figures for 65° and 85° were 1·46 and 1·26, showing the existence of an optimum temperature.

It is a striking fact that the acceleration of various vital processes produced by rise of temperature is very similar to that observed for ordinary chemical reactions. This is the case, for instance, with vegetable respiration.[2] Investigation of cherry-laurel leaves has shown that at 45° the output of carbon dioxide per unit weight of leaf is 0·0210 gram per hour, whereas at 16·2° the amount is only 0·0025 gram per hour. According to these figures, the temperature coefficient for a rise of 10° is 2·1, in good agreement with the value found for the temperature coefficient of chemical reactions generally. In regard also to assimilation of carbon dioxide at medium temperatures, the same relation exists between reaction velocity and temperature. Other cases where the temperature coefficient of velocity has been determined for changes in which living matter is involved are the development of sea-urchin and fish eggs,[3] the action of drugs on muscle,[4] and the conduction of

[1] *Zeit. physikal. Chem.*, 1901, 37, 475.
[2] Matthaei, *Phil. Trans.*, B, 1905, 197, 47.
[3] Abegg, *Zeit. Elektrochem.*, 1905, 11, 528; Herzog, *ibid.*, 820.
[4] Veley and Waller, *Proc. Roy. Soc.*, B, 1910, 82, 205.

an impulse along a nerve.[1] The value found for $\frac{k_{T+10}}{k_T}$ varies somewhat from case to case, but is in all instances of the same order as that found for chemical reactions.

[1] Lucas, *Journ. Physiol*, 1908, 37, 112.

CHAPTER XIV

ELECTROMOTIVE FORCE

REFERENCE has been made in an earlier chapter (pp. 158–9) to the circumstances in which a potential difference may originate at the common surface of two salt solutions or at the surface of a membrane bathed by an electrolyte. Such potential differences, although of importance in the interpretation of electro-physiological phenomena, are usually of a small order of magnitude. They are much smaller, as is well known, than the differences of electric potential which exist at the surface of a metal immersed in a salt solution. Now, although the conjunction of metal and salt solution does not occur with physiological fluids in their natural condition, it is found that valuable information regarding the nature of these fluids may sometimes be obtained by bringing them in contact with an electrode and measuring the potential difference which is developed. In order to understand this method of investigating physiological fluids, it will be necessary to consider a little in detail what are the conditions of equilibrium between an electrode and the solution in which it is immersed, and what are the factors which determine the potential difference.

Electrolytic Solution Tension and Potential Difference.—It is customary to regard a metal as possessing a certain tendency to give off positively charged ions when it is immersed in water or in an aqueous solution. The

magnitude of this tendency, the electrolytic solution tension, as it is called, varies from one metal to another: it is high, for instance, in the case of zinc; it is low for copper. So great is the difference in the electromotive behaviour of these two metals, that whereas a rod of zinc, immersed in a solution of zinc sulphate, gives off positive metallic ions to the solution and is itself left negatively charged, a rod of copper, immersed in a solution of copper sulphate, assumes a positive charge, owing to the deposition on it of positive ions from the salt solution. In this latter case, the feeble electrolytic solution tension of the copper is overcome by the osmotic pressure of the corresponding metallic ions in the salt solution, and some of these latter are deposited on the metal. It will be plain, however, that in any case the discharge of metallic ions into the solution, or the deposition of ions from the solution on the metal, can take place only to a very limited extent, owing to the electrostatic forces which come into play between the separated positive and negative electricity.

From the point of view just described it will be seen that the potential difference between metal and solution depends on and is determined by the relative value of the electrolytic solution tension of the metal on the one hand and the osmotic pressure of the metallic ions in the solution on the other hand. The exact relationship between the potential difference E, the solution tension P of the metal, and the osmotic pressure p of the metallic ions in the solution is established by thermodynamics, but the proof cannot be given here: it must suffice to state the formula and explain its significance. If T is absolute temperature and n is the valency of the metal under consideration, then $E = \frac{RT}{96540n} \cdot \log_e \frac{P}{p}$, R being the gas constant (see p. 10). By making a change to ordi-

nary logarithms, expressing R as volt-coulombs (*i.e.* $R = \cdot 082 \times 101 \cdot 8$, since 1 litre-atmosphere = 101·8 volt-coulombs), and taking $T = 290°$, corresponding to an average room temperature of 17° C., we have the simplified expression $E = \frac{0 \cdot 058}{n} \cdot \log_{10} \frac{P}{p}$. This is a fundamental formula expressing the potential difference (in volts) at the junction metal | salt solution, in terms of the valency of the metal, the osmotic pressure of the metallic ions in the solution, and the electrolytic solution pressure of the metal.

From the standpoint of the biologist or physiologist, the most significant feature of the foregoing formula is the presence in it of the quantity p, the osmotic pressure of the metallic ions in the solution which bathes the metal electrode. For it follows that the concentration of the ions in the solution is a factor in determining the potential difference, and a little consideration of the formula enables one to draw the quantitative conclusion that for every tenfold increase or decrease in the osmotic pressure of the metallic ions there is a change in the potential difference amounting to ·058 volt for a univalent metal, and $\frac{\cdot 058}{2}$ volt for a divalent metal.

A first illustration of the part played by the osmotic pressure of the metallic ions in determining the potential difference at a metal electrode may be taken from the well-known Daniell cell. This consists of a zinc rod immersed in a solution of zinc sulphate, separated by a porous pot from a solution of copper sulphate in which a copper electrode dips; when the zinc and copper poles are connected externally with a metal wire, a current flows through the wire from the copper to the zinc. In the Daniell cell, represented conveniently as Zn | $ZnSO_4$, $CuSO_4$ | Cu, there are, as in all ordinary galvanic cells,

two places where the junction metal | solution occurs, and the potential difference at each of the two electrodes can be expressed by the above formula. Neglecting the slight potential difference which arises at the common surface of the two solutions, we can express the E.M.F. of the Daniell cell as $E_1 - E_2$, where E_1 and E_2 are the potential differences at the zinc and copper electrodes respectively. Now, if P_1 and P_2 are the electrolytic solution tensions of zinc and copper, while p_1 and p_2 are the osmotic pressures of zinc and copper ions respectively in the two solutions, then $E_1 - E_2 = \frac{\cdot 058}{2} \log_{10} \frac{P_1}{p_1} - \frac{\cdot 058}{2} \log_{10} \frac{P_2}{p_2}$ $= \frac{\cdot 058}{2} \log_{10} \frac{P_1 p_2}{P_2 p_1}$, and the E.M.F. of the Daniell cell is accordingly given by this expression. It is easy to extract from this formula the conclusion that by diminishing p_1 or increasing p_2, *i.e.* by diluting the zinc sulphate solution or concentrating the copper sulphate solution, the E.M.F. of the Daniell cell should be raised. Experiment shows that this is actually the case, and so far, at least, the osmotic theory of galvanic cells is confirmed.

Concentration Cells.—From the standpoint adopted in this volume, one of the most interesting types of galvanic cell is the so-called "concentration cell," and the application of the osmotic theory to this case is of great importance. A concentration cell is to be conceived as one in which the two electrodes are of the same metal, and each electrode is bathed by a solution containing the corresponding metallic ions, the ion concentrations round the two electrodes, however, having different values. In such a cell there are three places at which differences of potential originate: (1) at the junction metal | dilute solution; (2) at the junction metal | concentrated solution; (3) at the common surface of the two solutions. In the case of concentration cells, the E.M.F. of which is

generally small, the potential difference between the two solutions cannot so lightly be neglected as in the case of the Daniell cell above. Whilst the potential difference between the two solutions can be evaluated, as shown by Nernst and Planck, it is customary in practical determinations[1] of the E.M.F. of concentration cells to eliminate it altogether by interposing between the two electrode solutions a concentrated solution of potassium chloride or potassium nitrate. For reasons which cannot be discussed here, this procedure practically gets rid of the potential difference between the two electrode solutions, so that the measured E.M.F. of the cell may then be taken as compounded of the two electrode potentials. In what follows it will be assumed that the liquid potential has been eliminated in the way described, and may therefore be neglected.

Suppose now that we have a concentration cell with two silver electrodes dipping in two solutions of silver nitrate, the osmotic pressure of the silver ions in the stronger solution being p_1, in the weaker solution p_2. A little consideration will show that when the silver electrodes are connected externally by a wire, current must flow *in the cell* from the weaker solution to the stronger, for the working of the cell must tend to diminish the difference in the concentrations of the electrode solutions. If E represents the electromotive force of this silver nitrate concentration cell, then $E = \cdot 058 \log_{10} \frac{P}{p_2} - \cdot 058 \log_{10} \frac{P}{p_1} = \cdot 058 \log_{10} \frac{p_1}{p_2}$; as the two electrodes are of the same metal, the electrolytic solution tension disappears from the formula. The electromotive force of such a cell is thus seen to be determined simply

[1] For a description of the usual compensation method of determining the E.M.F. of galvanic cells, a text-book of practical physical chemistry should be consulted.

by the ratio of the osmotic pressures of the ions in the electrode solutions; and if it is borne in mind that osmotic pressure is proportional to concentration, we may write $E = \cdot 058 \log_{10} \frac{c_1}{c_2}$, where c_1 and c_2 are the ion concentrations in the stronger and weaker solutions respectively.

With the help of the formula just developed, it is easy to calculate the electromotive force of a silver nitrate concentration cell, such, for instance, as the one represented by $Ag \mid \frac{N}{10}AgNO_3, \frac{N}{100}AgNO_3 \mid Ag$. The concentrations of the silver nitrate in the two electrode solutions are 0·1 and 0·01 respectively, but in order to get the values of c_1 and c_2, the concentrations of the silver ion, a knowledge of the degrees of electrolytic dissociation in $\frac{N}{10}AgNO_3$ and $\frac{N}{100}AgNO_3$ is necessary. From conductivity measurements these are found to be 0·82 and 0·94 respectively, so that $c_1 = 0{\cdot}1 \times 0{\cdot}82$, and $c_2 = 0{\cdot}01 \times 0{\cdot}94$. Hence $E = \cdot 058 \times \log_{10} \frac{\cdot 082}{\cdot 0094} = \cdot 055$ volt, a value which is in good agreement with the experimentally determined figure.

Just as for silver concentration cells, so also for the case of other univalent metals, $E = \cdot 058 \log_{10} \frac{c_1}{c_2}$. It is easily seen that if the electromotive force of a concentration cell of this type has been determined, and if the ion concentration round one electrode is known, the ion concentration round the other electrode can be calculated. This principle has found useful application in the determination of very small ion concentrations, and has been employed, for instance, in finding the solubility of sparingly soluble salts. As an illustration of the application of the principle, the problem of finding the solubility of silver iodide may be taken. For this

purpose a concentration cell represented by the scheme $Ag \left| \begin{matrix} AgI \\ + \\ KNO_3 \end{matrix} , \begin{matrix} \frac{N}{1000} AgNO_3 \\ + \\ KNO_3 \end{matrix} \right| Ag$ is set up, the potassium nitrate being added in order to diminish the resistance of the cell, and to eliminate the liquid potential, as already described. The solution round one electrode is $\frac{N}{1000} AgNO_3$, and, as this is very dilute, the silver ion concentration

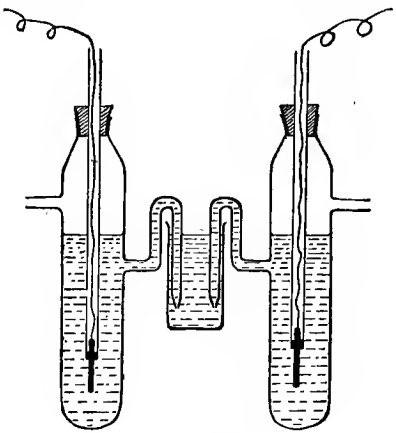

FIG. 24.

in this solution may be taken as ·001, the electrolytic dissociation being practically complete. The other electrode is bathed by a saturated solution of silver iodide—a solution in which plainly the silver ion concentration is exceedingly small. Experiment shows that the E.M.F. of the concentration cell just described is 0·22 volt, so that we have $0{\cdot}22 = {\cdot}058 \log_{10} \frac{{\cdot}001}{c_2}$, where c_2 is the concentration of the silver ions in the saturated solution of silver iodide. This equation gives $c_2 = 1{\cdot}6 \times 10^{-8}$ equivalents per litre, and since the dissociation of the silver

iodide may be taken as complete at such a great dilution, the figure $1{\cdot}6 \times 10^{-8}$ represents also the concentration of silver iodide in its saturated solution, and that is simply the solubility of the salt.

In setting up concentration cells and in the determination of electrode potentials generally, it is convenient to use separate electrode vessels, which can each be charged with their own particular solutions and then connected by means of an intermediate vessel. The foregoing Fig. 24 shows two electrode vessels which are in liquid connection with the contents of an intermediate beaker; the latter contains a strong solution of potassium nitrate or potassium chloride, in order to eliminate the potential difference between the electrode solutions, as already described.

The Hydrogen Electrode and its Applications.—In the foregoing discussion of concentration cells, it has been implied that the electrodes are invariably of metal and are the scene of a reversible equilibrium between a metal and its ions in the surrounding solution. This conception, however, must be extended to cover cases where the electrode substance is really a gas, this being in contact with a solution containing the same substance in the ionised condition. Since hydrogen is the gas which has chief significance in this connection, it is desirable to refer to it more especially, and, first of all, to describe the hydrogen electrode.

The vessel which is to serve in the construction of a hydrogen electrode is similar to one of those depicted in Fig. 24, but must be modified so as to permit a current of hydrogen gas to be bubbled through the solution: the modification consists in sealing on a narrow tube at the bottom of the electrode vessel. The electrode itself is a piece of platinum foil coated with platinum black (see

p. 125), or a thin film of platinum on glass, in either case saturated with hydrogen gas and half immersed in an acid solution, *i.e.* a solution containing hydrogen ions. When sufficient time has been allowed for the solution and the platinum to become completely saturated with the gas, this "hydrogen electrode" has a perfectly definite and steady potential, the numerical value of which is defined by the pressure of the hydrogen gas and the osmotic pressure of the hydrogen ions in the surrounding solution.

In like manner one may construct a chlorine electrode or an oxygen electrode. In the latter case the platinum foil or film, saturated with oxygen, is immersed in an alkali solution, which may be regarded as containing oxygen ions, derived from the hydroxyl ions which are mainly present, thus: $2OH' \rightleftarrows O'' + H_2O$.

Reverting to the hydrogen electrode, it is easily seen that, on the basis of the parallelism between this electrode and the metal electrodes already described, concentration cells may be constructed, the E.M.F. of which will be determined solely by the relative concentration of the hydrogen ion round the two electrodes. That is, if c_1 and c_2 are the concentrations of the hydrogen ion in the stronger and weaker acid solutions at the electrodes of a hydrogen concentration cell, the electromotive force of the cell at 17° C. is given by the formula $E = \cdot 058 \log_{10} \frac{c_1}{c_2}$, supposing that the potential at the common surface of the two solutions has been eliminated. This formula not only allows the calculation of the electromotive force of a hydrogen concentration cell from the concentrations of the hydrogen ion in the two electrode solutions—a calculation that is verified experimentally—but permits the evaluation of the hydrogen ion concentration in the one electrode solution, provided the electromotive force

of the cell and the hydrogen ion concentration in the other electrode solution are known. In order, therefore, to find the hydrogen ion concentration in any given liquid, the latter is made one of the electrode solutions in a hydrogen concentration cell, while dilute hydrochloric acid of known strength is taken for the other electrode solution. This method is particularly suited for determining very small hydrogen ion concentrations, for, the greater the difference between c_1 and c_2, the higher is the electromotive force of the concentration cell.

As a first example of the application of this method of finding the hydrogen ion concentration in aqueous solutions, we may take the problem of determining the ionisation of water.[1] It has been already explained (p. 259) that the application of the law of mass action to the equilibrium between water and its ions leads to the result that if C_H and C_{OH} represent the concentrations of the hydrogen and the hydroxyl ions respectively in any aqueous solution, then $C_H.C_{OH}=\text{const.}$ Now, one method of getting the value of this "ionic product" for water depends on the measurement of the E.M.F. of the hydrogen concentration cell represented by the scheme: $H_2\left|\frac{N}{100}HCl\right|\frac{N}{100}NaCl\left|\frac{N}{100}NaOH\right|H_2$. In this arrangement $H_2\left|\frac{N}{100}HCl\right.$ and $H_2\left|\frac{N}{100}NaOH\right.$ represent the hydrogen electrodes, the two electrode solutions being connected through an intermediate solution of sodium chloride. The latter is introduced in order to avoid the troublesome calculation of the potential difference which would arise if the $\frac{N}{100}HCl$ and $\frac{N}{100}NaOH$ were directly in contact. The potentials at the junctions of $\frac{N}{100}HCl$ with $\frac{N}{100}NaCl$,

[1] See Löwenherz, *Zeit. physikal. Chem.*, 1896, 20, 284.

and of $\frac{N}{100}$NaCl with $\frac{N}{100}$NaOH on the other hand, can be calculated easily with the help of the Nernst-Planck formula,[1] and at 25° are respectively ·0307 volt and ·0152 volt. In the hydrogen concentration cell under consideration, the positive current flows inside the cell from the alkali solution to the acid solution, and the total E.M.F. of the cell, determined by actual measurement, is 0·5378 volt at 25°. The two liquid potentials referred to are both opposed in direction to the electrode potentials, so that if E represents the E.M.F. compounded of the electrode potentials alone, $E = 0{\cdot}5378 + 0{\cdot}0307 + 0{\cdot}0152 = 0{\cdot}5837$ volt. But at 25°[2] $E = {\cdot}059 \log_{10} \frac{c_1}{c_2}$, where c_1 is the concentration of hydrogen ion in $\frac{N}{100}$HCl, and c_2 is the concentration of hydrogen ion in $\frac{N}{100}$NaOH; and since conductivity measurements show that hydrochloric acid in $\frac{N}{100}$ solution is electrolytically dissociated to the extent of 97·6 per cent., $c_1 = 0{\cdot}01 \times 0{\cdot}976 = {\cdot}00976$. Hence we have $0{\cdot}5837 = {\cdot}059 \log_{10} \frac{{\cdot}00976}{c_2}$, from which it follows that $c_2 = 1{\cdot}257 \times 10^{-12}$. The degree of dissociation of sodium hydroxide in $\frac{N}{100}$ solution is 0·935, so that the concentration of the hydroxyl ions in $\frac{N}{100}$NaOH is ·00935. Values have now been obtained for the concentrations of both hydrogen and hydroxyl ions in $\frac{N}{100}$NaOH, and the ionic product for this solution $= C_H . C_{OH} = 1{\cdot}257 \times 10^{-12} \times {\cdot}00935 = 1{\cdot}2 \times 10^{-14}$. According to the law of mass

[1] *Ann. Physik*, 1890, 40, 561.

[2] The higher temperature involves an increase in the coefficient of $\log_{10} \frac{c_1}{c_2}$, the value ·058 having been deduced for 17° C.

action, the value of the product $C_H.C_{OH}$ at a given temperature is the same in water as in any aqueous solution, and, since in pure water $C_H = C_{OH}$, it follows that the concentration of hydrogen ion in pure water and the concentration of hydroxyl ion in pure water are each given by $\sqrt{1{\cdot}2 \times 10^{-14}}$, that is, $1{\cdot}1 \times 10^{-7}$.

The example just discussed in detail shows clearly the utility of the hydrogen concentration cell in the determination of minute concentrations of hydrogen ion. For this purpose the electrometric method has the advantage over other methods (see, for example, pp. 167–8), which are adapted rather to the measurement of larger concentrations of the ion in question. The extent of hydrolytic dissociation of a salt, for instance, is a quantity that can readily be ascertained by the electrometric method. Suppose it were desired to find the extent of hydrolysis—in other words, the concentration of the hydroxyl ion—in $\frac{N}{1000}$ sodium acetate solution. This can be done by setting up the concentration cell represented by the scheme: $H_2 \mid \frac{N}{1000}$ HCl $\mid \frac{N}{1000}$ NaCl $\mid \frac{N}{1000} CH_3COONa \mid H_2$, determining the E.M.F. of this cell, and then calculating the hydrogen ion concentration in the $\frac{N}{1000}$ CH_3COONa as already described. When the hydrogen ion concentration has thus been ascertained, that of the hydroxyl ion can easily be calculated, for, as shown in the last paragraph, the product of the two concentrations, $C_H \times C_{OH}$, has a constant value, which at 25° is $1{\cdot}2 = 10^{-14}$.

The first instance of the application of the foregoing electrometric method in connection with more definitely physiological problems is furnished by Bugarszky and Liebermann's work [1] on the relation between protein and

[1] *Pflüger's Arch.*, 1898, **72**, 51.

electrolytes. The acid-alkali concentration cell described on p. 316 was employed in this investigation, and the effect of adding protein either to the acid or the alkali was studied quantitatively. In this way definite information was obtained as to the influence of protein on the concentration of hydrogen ion in a given acid solution and on the concentration of hydroxyl ion in a given alkali solution. The results showed that albumin has the power of combining both with acid and with alkali.[1]

Concentration of Hydrogen Ions in Physiological Fluids.—The determination of the exact degree of acidity or alkalinity of a physiological fluid by the ordinary titration methods is not an easy matter. In these circumstances the measurement of the hydrogen ion concentration by the electrometric method furnishes valuable information. Blood has frequently been examined in this way,[2] and by the determination of the E.M.F. of such concentration cells as $H_2 \left| \frac{N}{100} HCl + \frac{N}{8} NaCl \right| \frac{N}{8} NaCl \left| Blood \right| H_2$, it has been shown that the concentration of hydrogen ion in fresh defibrinated mammalian blood is $0{\cdot}3 \times 10^{-7} - 0{\cdot}7 \times 10^{-7}$ at ordinary temperature. Since the concentration of hydrogen ion in water at ordinary temperature is $0{\cdot}8 \times 10^{-7}$, it appears that defibrinated mammalian blood is practically a neutral liquid. It is worth noting, however, that if in the measurement of the E.M.F. of the gas cell, a current of hydrogen is passed through the blood, with the result that the carbon dioxide normally present in this fluid is removed, then a distinctly lower value, viz., $0{\cdot}01 \times 10^{-7} - 0{\cdot}03 \times 10^{-7}$, is obtained for the hydrogen ion concentra-

[1] See p. 265, and cp. Robertson, *J. Physical Chem.*, 1910, **14**, 528.

[2] Höber, *Pflüger's Arch.*, 1900, **81**, 522; 1903, **99**, 572; Michaelis and Rona, *Biochem. Zeit.*, 1909, **17**, 317; Hasselbalch, *ibid.*, 1910, **30**, 7.

tion. This figure corresponds with a feebly alkaline reaction. Rise of temperature also appears to favour alkalinity, for electrometric measurements, similar to the above but carried out at 37–38°, indicate that at body temperature the concentration of hydroxyl ions in the blood is somewhat greater than at ordinary temperature.

The examination of other body fluids on the same lines as those described for blood has confirmed the earlier conclusion that in the case of the higher animals these fluids are generally neutral. Those which exhibit a notable departure from neutrality are gastric juice, pancreatic juice, intestinal juice, and urine. Hydrogen cell measurements have shown that in the case of gastric juice C_H (concentration of hydrogen ion) has the value $3 \times 10^{-2} - 9 \times 10^{-2}$, whilst in the pancreatic juice and the intestinal juice $C_H = 7 \times 10^{-10} - 11 \times 10^{-10}$. The acidity of the urine, even for a single individual, varies within wide limits, and the value of C_H may be put down as $1 \times 10^{-7} - 1 \times 10^{-5}$.

Reference has already been made to the difficulty of ascertaining the acidity or alkalinity of a physiological fluid by the ordinary titration methods. These methods involve the use of indicators, and it is a well-known fact that many indicators undergo change of colour before the point of absolute neutrality (*i.e.* $C_H = C_{OH}$) is reached. Thus, for instance, the turning point lies at $C_H = 10^{-1.4} - 10^{-2.6}$ for tropäolin OO, at $C_H = 10^{-3.1} - 10^{-4.4}$ for methyl orange, at $C_H = 10^{-5.0} - 10^{-7.0}$ for *p*-nitrophenol, and at $C_H = 10^{-8.3} - 10^{-10}$ for phenolphthalein.[1] Provided, however, that the turning point, in terms of hydrogen ion concentration, is known for each of a large number of indicators, then with the help of such a "set" of indicators the degree of acidity or alkalinity of a liquid not far from the point of neutrality could be ascertained with

[1] See Sörensen, *Biochem. Zeit.*, 1909, 21, 131; 22, 352; 1910, 24, 381.

fair accuracy. For the establishment of such a scale of indicators it is necessary to have invariable and reproducible standards of acidity and alkalinity, covering more especially the range from $C_H = 1 \times 10^{-3}$ to $C_{OH} = 1 \times 10^{-3}$. For this purpose the solutions obtained by mixing sodium hydroxide and phosphoric acid in different proportions are of great importance,[1] the exact degree of acidity or alkalinity of each such solution being determined and controlled electrometrically. In this way it has been found that for $\frac{N}{10}NaH_2PO_4$ the value of C_H is $1{\cdot}2 \times 10^{-4}$, while for $\frac{N}{10}Na_2HPO_4$ the value of C_H is $1{\cdot}4 \times 10^{-9}$.

With a suitable "set" of indicators available, it becomes possible to ascertain the degree of acidity or alkalinity of a liquid, and Sörensen (*loc. cit.*) has investigated a number of physiological fluids by this colorimetric method. The method, however, must be used with caution, inasmuch as some indicators behave abnormally in presence of proteins or neutral salts. It is, after all, on physico-chemical measurements that one depends for accurate and trustworthy determinations of the concentration of the hydrogen ion in physiological fluids.

[1] See Prideaux, *Biochem. Journ.*, 1911, 6, 122.

SUBJECT INDEX

AUTHOR INDEX

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